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M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 1. Sensory adaptation to weightlessness and readaptation to one-g: an overview

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Summary. Experiments on human spatial orientation were conducted on four crewmembers of Space Shuttle Spacelab Mission 1. This introductory paper presents the conceptual background of the project, the relationship among the experiments and their relevance to a "sensory reinterpretation hypothesis". Detailed experiment procedures and results are presented in the accompanying papers in this series. The overall findings are discussed in this article as they pertain to the following aspects of hypothesized sensory reinterpretation in weightlessness: 1) utricular otolith afferent signals are reinterpreted as indicating head translation rather than tilt, 2) sensitivity of reflex responses to footward acceleration is reduced, and 3) increased weighting is given to visual and tactile cues in orientation perception and posture control. Three subjects developed space motion sickness symptoms, which abated after several days. Head movements, as well as visual and tactile cues to orientation influenced symptoms in a manner consistent with the sensory-motor conflict theory of space motion sickness. Six short duration tests of motion sickness susceptibility, conducted pre-flight, failed to predict sickness intensity in weightlessness. An early otolith-spinal reflex, measured by electromyography from the gastrocnemius-soleus muscles during sudden footward acceleration, was inhibited immediately upon entering weightlessness and declined further during the flight, but was unchanged from pre-flight when measured shortly after return to earth. Dynamic visual-vestibular interaction was studied by measuring subjective roll self-motion created by looking into a spinning drum. Results suggest increased weighting of visual cues and reduced weighting of graviceptor signals in weightlessness. Following the 10 day flight, erect posture with eyes closed was disturbed for several days. Somewhat greater visual field dependence post-flight was observed for two of the crew. Post-flight tests using horizontal linear acceleration revealed an increased variance in detection of acceleration. The ability of the returned crew to use non-visual lateral acceleration cues for a manual control task appeared enhanced over their pre-flight ability for a few days after return.

Key words: Spatial orientation – Vection – Motion sickness – Vestibular – Weightlessness

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Introduction

The nearly weightless (*microgravity*) environment of spaceflight provides challenging opportunities for research on sensory-motor adaptation. This paper provides an introduction to the series of interrelated experiments performed on the first Spacelab mission (SL-1) in November 1983 by a team of investigators from MIT and Canada. These investigations, most of which are described in detail in the accompanying five articles, are all aimed at assessing human vestibular and visual responses in space and are intended to clarify the presumed alteration in sensory and motor function in weightlessness. Our working hypothesis, which tied together the various experiments and against which the results are tested, is one of "sensory reinterpretation." A preliminary report was published previously (Young et al. 1984).

Our experiments were designed to help assess human sensory/motor adaptation to weightlessness and readaptation to earth's gravity, and to simultaneously examine the question: is space sickness a motion sickness? The underlying neuroscience research question is how a fully developed sensory

motor system, which receives redundant information from several sensory mechanisms, reorganizes to account for the environmentally imposed change in the relationship between motor commands and sensory feedback. The results of this research relate to classic studies of sensory rearrangement (e.g. Held and Freedman 1963; Rock 1966; Wallach and Smith 1972; Wallach and Bacon 1976) and to recovery from vestibular lesions (e.g. Igarashi et al. 1970; Fregley and Graybiel 1970). In particular, we ask how pitch and roll perception and postural adjustment are affected by the abnormal pattern of otolith afferent signals which must accompany sustained weightlessness. Our working hypothesis, explained below, was that in the process of sensory adaptation to weightlessness, the low frequency components of the otolith afferent signals (dependent upon head orientation in 1-g) are centrally inhibited or reinterpreted, and that visual and tactile cues consequently play an increasing role in spatial orientation.

Our research also relates directly to the etiology of space sickness, now recognized as a significant problem impacting astronaut performance, safety and well-being. Although space sickness symptoms were not reported in the smaller Mercury and Gemini spacecraft, they have been consistently reported in the Soviet space program (Matsnev et al. 1983) and experienced by Apollo and Skylab crews (Homick and Miller 1975; Graybiel et al. 1977). The incidence among Shuttle crews has exceeded 50% (Homick et al. 1985). It has been parsimonious to assume that the genesis of space sickness is similar to that of motion sickness as experienced on earth (e.g. Benson 1977; Oman 1982b), although conclusive evidence has been lacking and alternative hypotheses have been suggested (see Oman et al., this issue). The etiology of motion sickness is thought to involve the same physiological mechanisms responsible for spatial orientation and body movement control. Based on a sensory-motor conflict theory (Reason 1978; Oman 1982a), motion sickness results when incoming sensory signals no longer match expected patterns learned during previous sensory/motor experience. Because of the environmentally imposed change in graviceptor response to head movements in weightlessness, motion sickness was expected to occur in space. Space sickness would be expected to be exacerbated by real or perceived changes in body orientation, and to subside with a time course paralleling adaptation of sensory-motor systems subserving spatial orientation.

Earlier formal space flight investigations of the influence of weightlessness on human vestibular responses have included the pioneering studies of Graybiel and coworkers (1977) who observed the absence of motion sickness susceptibility to out of plane head movements made in a rotating chair when tested after the fifth day in space. They also showed the ability to maintain a body oriented reference frame in weightlessness. Homick and Reschke (1977) reported postural instabilities with eyes closed following return of the Skylab astronauts to earth. Other tests of inflight postural stability (Clement et al. 1984) and assessment of the vestibuloocular and optokinetic reflexes have been conducted more recently (Thornton et al. 1985; Watt et al. 1985; Vieville et al. 1986). Relevant Soviet research on man in space has largely been limited, until quite recently, to assessment of motion sickness countermeasures, relationship of spatial illusions to symptoms, and post-flight studies of orientation perception, neuromuscular function and ocular counterrolling (e.g. Yakovleva et al. 1980; Matsnev et al. 1983). Spacelab-1 provided the opportunity for three teams of experimenters (European Space Agency, NASA Johnson Space Center, and MIT/Canada) to perform extensive tests on vestibular function of the same crewmembers during a mission devoted to scientific goals.

A sensory reinterpretation hypothesis for adaptation to weightlessness and readaptation to one-g

A sensory reinterpretation hypothesis formed the basis for our proposed experiments and serves as a useful tool for interpreting the results (Young et al. 1983). It assumes that the functionally appropriate physiological adaptation to weightlessness should involve a reinterpretation of afferent signals originating in the graviceptors, particularly in the otolith organs. These receptors act as linear accelerometers, and respond to the physical input of gravitoinertial force. The adequate input to the otolith organs is the force per unit mass or "specific force" (f), familiar to users of accelerometers for inertial navigation (Fernandez and Macomber 1962). This force, acting on the otolithic membranes, is equal to the vector sum of gravity (g) minus linear acceleration (a). Physically, specific force is the entity tracked by a pendulum. On earth, a non-accelerating body is subject only to the "downward" specific force vector g, and the pendulum points toward the vertical. In orbital flight, a body which is not accelerating relative to the spacecraft experiences a linear acceleration a (as the spacecraft free falls around the earth) equal to the gravitational acceleration g. The specific force acting on the otolith organs is zero, except when head movements are made. Disregarding small gravity gradient effects, a pendulum in earth orbit would assume any arbitrary orientation and velocity previ-

ously imparted to it, and would be of no use in indicating the direction of the center of the earth, or of the spacecraft floor. The otolith organs, of course, continue to provide the central nervous system (CNS) with afferent signals which are modulated by each head acceleration. We believe that on earth the signals from the saccular as well as the utricular otolith organs serve a dual function in spatial orientation and posture control – to estimate the static orientation of the head with respect to the vertical (the traditional graviceptor function) and also to estimate the linear acceleration of the head during movement. The potential ambiguity in interpretation of otolith signals (tilt vs. acceleration) is presumably resolved by CNS integration of information from the semicircular canals, other orientation senses, and knowledge of commanded motion, based on sensory-motor experience in the prevailing environment. In general, the lower frequency components of the otolith signals indicate the direction of the head relative to gravity, whereas the higher frequency components reflect both head tilt and linear acceleration.

In space, where static head orientation doesn't influence otolith organ afferent activity, each head movement produces a specific force stimulus which can swing rapidly in direction even in the absence of any head tilt. The critical question, for which space experiments are necessary, is whether the CNS adapts to accept a radically new relationship between otolith afferent signals and static and dynamic body movement – as appropriate to the new environment. If such adaptation takes place, its time course and its relationship to space motion sickness become important. The removal of a 1 g bias could, in itself, shift the otolith organs to a new portion of their nonlinear operating range, thereby altering their utility in responding to accelerations. One possibility is that the otolith signals are largely inhibited, reducing their influence on posture, eye movements and spatial orientation, and consequently leading to a decrease in the ability to sense linear acceleration of even a transient nature. An alternative hypothesis is that otolith signals are reinterpreted as the CNS learns – via sensory-motor interactions with the weightless environment – that the afferent signals now code only linear acceleration. This hypothesis assumes a robust adaptive capacity and is consistent with much previous research on adaptation to other specific sensory rearrangements (reviewed by Welch 1978). Similar hypotheses have been put forth by other groups (von Baumgarten et al. 1981; Parker et al. 1985). All of our experiments in this program were aimed in one way or another at testing this hypothesis (Oman 1982; Young 1983).

Spacelab-1 mission operations

Spacelab-1 was the first flight of the Spacelab pressurized module, a 30 foot long, manned laboratory for scientific and technical research developed by the European Space Agency (ESA) and carried into orbit in the cargo bay of the Space Shuttle. The "payload crew" of four, which performed all experiments, consisted of two NASA Astronaut Mission Specialists (one of whom had previous Skylab flight experience) and two Payload Specialists chosen by the investigators from the outside scientific community. One of the Payload Specialists was BKL, a vestibular researcher and bioengineer from our MIT laboratory. The Commander and the Pilot did not participate in flight or pre/post-flight experiments. Subjects were male, ranged in age from 35 to 53 years, and were active pilots. They were in good health and were examined and judged normal by our consulting otoneurologist. To preserve anonymity and facilitate data comparison, these subjects are referred to only by letter code A–D throughout this issue. Two crew pairs (A and B, C and D) worked alternating 12 h shifts throughout the mission. Crew circadian rhythms were shifted beginning 14 days before launch, with only partial success. After landing, circadian cycles were abruptly shifted back to local time. It was not possible to control for circadian effects in our testing.

During Spacelab missions, the payload crew lives in the Orbiter and works in the Spacelab, commuting via an access tunnel. The laboratory is maintained at normal sea level atmospheric pressure and air composition, and at comfortable temperature and humidity. Conduct of the scientific mission was substantially different from any flown previously. The investigators on the ground and their astronaut colleagues participated in extensive training, simulation and discussion of scientific goals. They performed as an integrated team, facilitated during the mission for the first time by frequent TV coverage and two-way voice communication. This flexibility permitted numerous repairs and adjustments of experiments (Garriott et al. 1984). Despite the flexibility introduced in Spacelab-1 relative to previous missions, the conduct of experiments was severely restricted in comparison to a normal ground laboratory. The competition for crew time, power, communications and other resources, and the relatively short mission duration prevented substantial extension of measurements.

For this first mission, a wide variety of experiments from the U.S., Canada, eleven European countries and Japan were included (Chappell and Knott 1984). The three closely related sets of vestibu-

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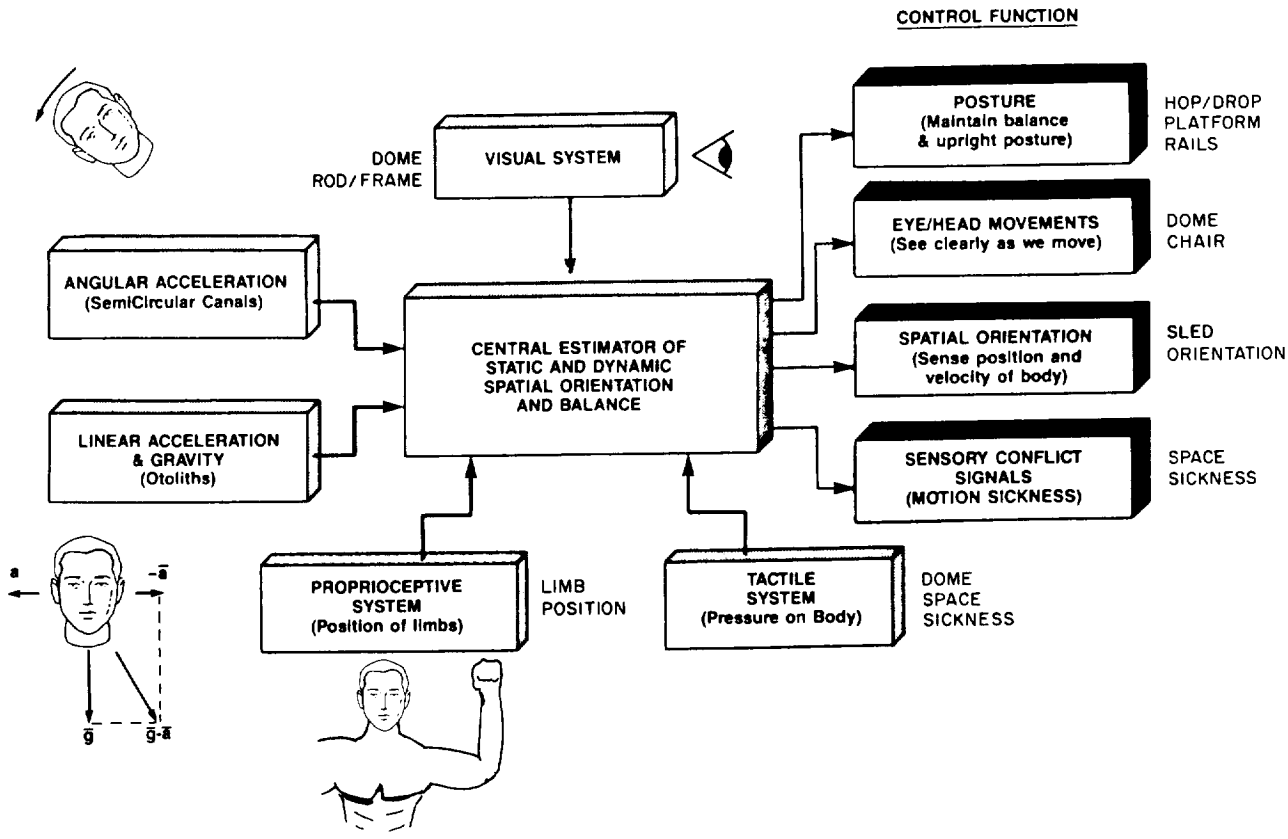

Fig. 1. Scope of the MIT/Canadian Spacelab 1 experiments, by experiment short name, relative to a schematic representation of the role of the vestibular and other senses in control of posture, eye movements and perception of orientation. Experiment short names are keyed to Table 1

lar investigations (von Baumgarten et al. 1984; Reschke et al. 1984; Young et al. 1984) required considerable crew flight time and dominated the pre- and post-flight testing.

Spacelab-1 was launched on November 28, 1983 and was extended from a planned nine days to a mission lasting 10 days, 8 h, 47 min, with a landing at Edwards AFB, California. The landing was delayed by 8 h because of computer malfunctions, severely reducing the crew availability for post-flight testing on the landing day. The NASA nomenclature used for the flight and preserved in the accompanying articles designates the pre-flight days relative to launch. "L minus one" (L −1) is the day before launch. Flight days are numbered beginning with zero. Hence Mission Day 1, or MD1, is the second 24 h in orbit. Post-flight days also are numbered from zero (R+1 is one calendar day after the return day). Mission Elapsed Time (MET) is specified in days/hours : minutes since launch.

Scope and interrelationships of the experiments

The overall scope of our SL-1 experiments and their relationship to the stimuli and outputs of the human system for spatial orientation and balance is indicated in Fig. 1. Individual experiments, investigators and SL-1 performances are shown in Table 1. Each experiment examined a different output to reveal some aspect of the way the CNS adapts to the functional equivalent of removing the gravity vector. The "Rotating Dome" experiment explored central integration of conflicting visual/vestibular/tactile sensory cues by measuring roll self-motion and compensatory eye and head movements stimulated by looking into the open end of a rolling drum. The "Rod and Frame" is a pre-post flight test of static visual field dependence. The "Hop and Drop" experiment studied the otolith-spinal reflex which normally prepares one for a landing from a fall. Electromyographic activity from the gastrocnemius and soleus

Table 1. MIT/Canadian vestibular experiments on SL-1

Experiment	Lead investigator	When performed
1. Visual-vestibular interaction (dome)	Young	Subj. A, B, MD 1, 2, 4, 5, 6, 7; C, D, MD 1 (failed) 3, 6
2. Otolith-spinal reflex (hop/drop)	Watt	Subj. A, MD 0, 1, 6; B, MD 0, 1, 6, 7
3. Awareness of orientation and limb position	Money	*Subj. B, MD 1; C, MD 8
4. Posture control (platform/rails)	Kenyon	Pre-Postflight
5. Motion sickness susceptibility (space sickness)	Oman	Subj. A, B, C, D continuous
6. Perception of linear acceleration (sled)	Arrott	Pre-Postflight (sled scheduled for D-1)
7. Ocular torsion during lateral acceleration	Young	**Subj. C, D, MD 0, MD 7
8. Vestibulo-ocular reflex nystagmus dumping (chair)	Oman	*Subj. A, B, MD 7; C, MD 3, 6

All in-flight tests were also performed pre- and post-flight
MD: Mission Day
* Data still being analyzed – not reported in this issue
** No flight data available due to equipment failure. Full test scheduled for D-1. Pre-postflight data reported with expt. 6

muscles of the leg was measured during footward acceleration provided by stretched elastic cords. The "Position Awareness" experiment measured the influence of weightlessness on both the orientation of perceived objects in the absence of a vertical and the accuracy of proprioceptive cues in determining perceived limb position. The "Space Sickness" investigation clinically characterized space sickness symptoms and studied their relationship to head movements, visual, tactile and proprioceptive cues, and to the shift of body fluids toward the head. A "Posture Platform" and narrow rails were used to measure the post-flight degradation of postural stability. The "Sled" is a linear acceleration device which was used for stimulating eye deviation and ocular torsion, as well as subjective motion during horizontal linear acceleration. A rotating chair was used to stimulate the semicircular canals for study of the horizontal vestibulo-ocular reflex and the "dumping" of post-rotatory nystagmus produced by head pitch.

The experiments conducted on Spacelab 1 were the first of a planned series of related investigations, scheduled for continuation and extension on several additional Spacelab missions in the mid-eighties. For operational reasons the experiments originally planned for use with the Space Sled, a controlled linear acceleration device, were postponed until the D-1 Spacelab mission, accomplished in November, 1985. Related tests were performed on the 1984 Shuttle 41-G Mission (Watt et al. 1985).

Pre-flight testing of the crew for the MIT/Canadian experiments was conducted from 1979 through 1983 at the experimenters' laboratories (MIT, McGill, DCIEM/Toronto) and at NASA's Johnson Space Center, Kennedy Space Center, and Dryden Flight Research Facility (DFRF). Of particular value for protocol development, training and baseline data collection were the series of four sets of parabolic flight tests producing repeated 20–25 s periods of weightlessness in NASA's KC-135 aircraft. Pre-flight and post-flight testing by all life science experimenters was conducted at an especially constructed Baseline Data Collection Facility at DFRF at approximately 152, 122, 65, 44 and 10 days before launch. Subjects A and B were tested within hours of landing, and all four subjects were tested on 1, 2, 4 and 6 days after return. Parabolic flights to assess 0 g motion sickness susceptibility and reorientation illusions were performed pre-flight, and 3 days, and 1 year after landing.

Results and discussion

The results of our experiments on Spacelab 1, discussed in detail in the accompanying papers, must be interpreted cautiously because the experiments were conducted on only 2–4 subjects, and with fewer repetitions and frequently under less well controlled conditions than desired. These results, when taken together with findings from other related experiments, appear generally consistent with the sensory reinterpretation notion. We are aware of no evidence pointing to pathological alteration of sensory function at the end organ.

Early in the SL-1 flight 3 of 4 subjects developed space sickness symptoms, which largely resembled those of prolonged motion sickness, superimposed on the effects of fluid shift towards the head. Symptoms abated after 2–3 days. Short duration pre-flight motion sickness susceptibility tests did not predict in-flight sickness intensity. However, head movements, especially in pitch, as well as visual and tactile cues to orientation, influenced symptom level

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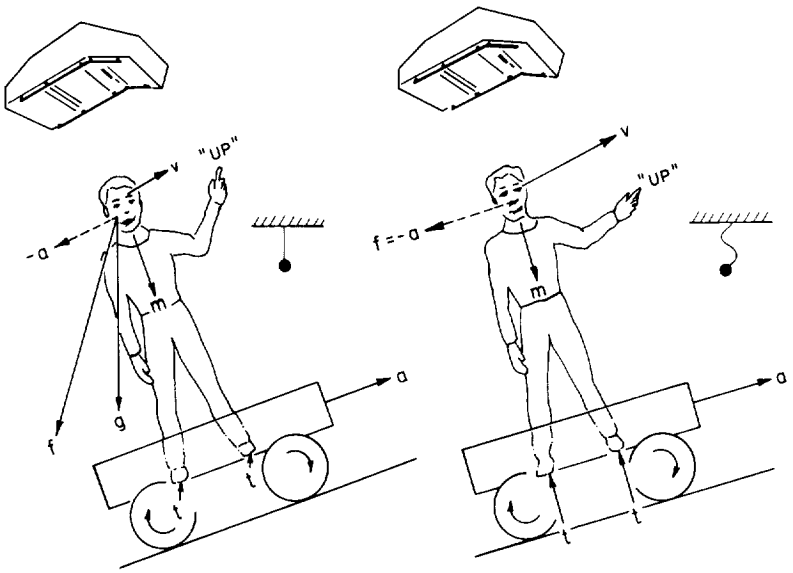

a. PRE-FLIGHT ORIENTATION VECTORS. b. IN-FLIGHT ORIENTATION VECTORS.

Fig. 2a, b. Schematic representation of the sensory vectors which are used in determining human spatial orientation. In **a**, the subjective zenith is arrived at by a vector sum of the various sensory contributions, but is dominated by the gravitoinertial vector (f). If the subject, shown standing on a moving wagon, were not accelerating, this would indeed be vertical (g). The subjective vertical is also biased slightly by the influence of vertical or horizontal elements in the visual field (v), by localized tactile cues (t), and by one's own body axis (m). The strength of these other cues depends on the individual. In **b**, which represents the similar situation in weightlessness, the crucial difference is that the gravitoinertial vector now represents only linear acceleration (**a**). The subjective zenith, or local reference axis if "up" has lost all meaning, now ignores the gravito-inertial vector in favor of the stronger visual, tactile and body centered axes. Tactile cues normal to support surfaces, such as illustrated in **b**, could be developed by a loading mechanism such as stretched elastic cords (not shown) or briefly by extension of the legs. Differences among the individual crew members in the relative strength of these vectors is reflected in the range of orientation styles

in ways consistent with the sensory conflict theory for motion sickness and with the hypothesis of sensory reinterpretation.

Changes in sensory-motor function were observed both during the flight and extensively following the landing. Otolith-spinal reflex responses to footward acceleration with head erect were inhibited when tested early in the flight, and declined further during the week in weightlessness. However, in the tests performed several hours after landing the otolith-spinal reflex had returned to pre-flight levels. Similarly, the short latency reflex reactions to destabilization of standing on the posture platform were unchanged post-flight, although the longer latency responses demonstrated postural instability, with eyes closed, on both the platform and on the rails tests. The Rotating Dome experiment data suggest increased weighting of visual cues and tactile cues, and reduced influence of graviceptor signals in determination of orientation in weightlessness. Post-flight measurements also suggested a slight increase in static visual field dependence. Proprioception may have been degraded in flight. Post-flight reaction to horizontal linear acceleration revealed a reduction in dynamic ocular counterrolling, and increased variability in the detection of low level accelerations, but an enhanced ability to use suprathreshold acceleration cues to null lateral position in a closed loop, non-visual, tracking task.

As illustrated in Fig. 2, the human estimation of body position and postural reactions is thought to change in weightlessness to make use of the varied sensory inputs in a manner which is fundamentally appropriate to the microgravity condition. In particular, it appears likely that at least three separate aspects of such reinterpretation may be present: tilt acceleration reinterpretation, reduced postural response to z-axis linear acceleration, and increased attention to visual cues. In the course of the reinterpretation, motion sickness symptoms, caused by the original sensory motor conflicts, gradually disappear.

As illustrated in Fig. 2a, for pre-flight spatial orientation, the subject relies heavily on the static gravitoinertial vector for his perception of the vertical, which can be displaced by a low frequency acceleration (e.g. Mach 1875; Howard and Templeton 1966; Schöne 1980; Young 1984). However, each individual has his perception of the upright influenced, to varying degrees, by the presence of elements in the visual field, especially those normally associated with the vertical (e.g. Witkin 1958; Howard 1982) and by localized tactile cues such as pressure on the soles of the feet. Moving visual scenes (not shown in the figure) can also create a sense of body self-motion. Furthermore, each individual has a tendency to align the perceived vertical toward the head or feet along the torso long axis.

This tendency is represented by an idiotropic body axis vector and is assumed to vary in strength among individuals (Mittelstaedt 1983).

These sensory vectors must be reinterpreted for spatial orientation in weightlessness. As shown in Fig. 2b, the gravitoinertial vector now is merely the opposite of linear acceleration relative to the spacecraft. If it were to continue to dominate the perception of tilt orientation, the astronauts would experience 180 degrees of roll or pitch each time they accelerated and decelerated while translating through the spacecraft, which was never reported. Instead, we believe that the signals from the graviceptors are reinterpreted to represent linear translation, as required for locomotion accuracy in space, and as carried over to the post flight closed loop acceleration nulling tests. In-flight postural reaction to changes in acceleration, at least along the body z-axis (Watt et al., this issue; Reschke et al., this issue) show a decrease in sensitivity, which is consistent with the absence of a need to prepare the "anti-gravity muscles" for a fall. (It remains to be determined whether this inhibition is limited to z-axis acceleration.) Upon return to earth this reinterpretation of graviceptor cues leads to a decreased ability to stand up with eyes closed, except within a very narrow cone of static stability near the upright. Actual head tilt may be perceived as a lesser tilt postflight, combined with linear acceleration in the opposite direction, leading to destabilizing postural reactions in the wrong direction. Post-flight changes in postural control strategy may be related to this tilt/translation reinterpretation (Kenyon and Young, this issue, Reschke et al. 1984). Ocular counterrolling, which is a normal compensatory response to a tilted gravitoinertial vector, is also shown to be reduced post-flight dynamically (Arrott and Young, this issue) and statically (von Baumgarten et al. 1984; Parker et al. 1985; but not Yakovleva et al. 1980). Post-flight perceived tilt, in the dark, is reduced (Benson et al. 1984) as predicted by the hypothesized carry-over of the otolith reinterpretation, and dynamic tilt was reported on other crews to lead to a strong translation sensation (Parker et al. 1985, who independently arrived at a similar otolith tilt/translation reinterpretation hypothesis).

In the absence of usable graviceptor information regarding body orientation in weightlessness, the nervous system must pay increased attention to the remaining sensory orientation signals. Subjective reports from crew members indicate large variations in individual styles, but never a prolonged sense of absence of a reference frame or "disorientation". The increased length of the "visual" vector in Fig. 2b is intended to represent the increased weighting given to dynamic visual inputs to self motion (the dome experiment) and to static elements such as the floor or ceiling, another crew member, or the earth (Oman et al., this issue). In many cases the relative weighting may be a complete domination by the visual, body control or tactile vector in weightlessness. Large individual differences in visual field influence in weightlessness are reflected in the post-flight increases in field dependence. Similarly, localized tactile cues, such as pressure on the feet in the Dome and the Hop/Drop experiments or on the buttocks and back when wedging into a corner, serve to take on an increasing role in determining spatial orientation and a sense of well-being. Finally, the influence of the postulated body-axis orientation vector, which could allow some crew members to orient their reference frame to their body long axis in weightlessness, is greater than pre-flight because of the reinterpretation of the graviceptor cues.

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Young LR, Oman CM, Watt DGD, Money KE, Lichtenberg BK (1984) Spatial orientation in weightlessness and readaptation to earth's gravity. Science 225: 205–208

Young LR, Shelhamer M, Modestino SA (1986) M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 2. Visual vestibular interaction in weightlessness. Exp Brain Res 64: 299–307

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SECTION II - ABSTRACTS

This section provides a reference source for additional information about the research that has been conducted by the SLS-1 investigator teams. Because all the investigators have published widely, space does not permit full presentation of all their published work. Instead, in addition to the selected papers presented in Section I, abstracts and bibliographic citations of additional papers by the investigators are presented in this section. These papers often contain more detailed information, or concentrate on more specific topics, than the overview papers in Section I.

Gmünder FK, and Cogoli A. Cultivation of single cells in space. ***Appl Micrograv Tech I*** **1988;3:115-122.** The purpose of this review is to present an updated and comprehensive analysis of the experiments with single cells performed in space. Especially the results of the investigations performed in Biorack on the D-1 mission clearly show that important cellular functions are changing in microgravity. Cell proliferation, differentiation, metabolism, membrane properties, and cytoplasmic streaming underwent significant alteration during exposure to space flight conditions in a variety of single cells cultures spanning from bacteria to mammalian cells. These findings open new and interesting perspectives to basic and applied research in microgravity. The focus of this paper is on the cultivation of mammalian cells in space laboratories and on the related instrumentation. While Biorack is a useful and efficient instrument for simple studies in Spacelab, the development of new facilities like incubators with automated fixation devices as well as of more complex bioreactors is strongly recommended.

Sieber-Blum M, Kumar SR, and Riley DA. In vitro differentiation of quail neural crest cells into sensory-like neuroblasts. ***Dev Brain Res*** **1988;39:69-83.** This study shows that quail neural crest cells can differentiate in vitro into sensory-like neuroblasts. The putative sensory neuroblasts were large and spherical, possessing large diameter, bipolar or pseudo-unipolar, long processes that lacked multiple varicosities characteristic of autonomic neurons. They bound HNK-1, a monoclonal antibody against a cell surface epitope expressed by early neural crest cells but not by young neural tube-derived cells. Many of the sensory-like neuroblasts had substance P (SP)-like immunoreactivity. Some exhibited histochemical carbonic anhydrase activity; carbonic anhydrase is shown in this study to stain a subpopulation of spinal sensory neurons in adult quail and embryos 9 days and older, whereas ventral root axons and neurons in sympathetic ganglia are non-reactive at all ages. Double staining indicated that unlike the multipolar neuroblasts developing in the same cultures, SP-like immunoreactive neuroblasts do not contain detectable levels of tyrosine hydroxylase or dopamine-ß-hydroxylase. Finally, the neuronal nature of the cultured sensory-like neuroblasts was further documented by double labeling for antibodies against the 68 kDa neurofilament polypeptide and substance P.

CELL BIOLOGY

NEUROVESTIBULAR

Arrott AP, and Young LR. M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 6. Vestibular reactions to lateral acceleration following ten days of weightlessness. *Exp Brain Res* 1986;64:347-357. Tests of otolith function were performed pre-flight and post-flight on the science crew of the first Spacelab Mission with a rail-mounted linear acceleration sled. Four tests were performed using horizontal lateral (y-axis) acceleration: perception of linear motion, a closed loop nulling task, dynamic ocular torsion, and lateral eye deviations. The motion perception test measured the time to detect the onset and direction of near threshold accelerations. Post-flight measures of threshold and velocity constant obtained during the days immediately following the mission showed no consistent pattern of change among the four crewmen compared to their pre-flight baseline other than an increased variability of response. In the closed loop nulling task, crewmen controlled the motion of the sled and attempted to null a computer-generated random disturbance motion. When performed in the light, no difference in ability was noted between pre-flight and post-flight. In the dark, however, two of the four crewmen exhibited somewhat enhanced performance post-flight. Dynamic ocular torsion was measured in response to sinusoidal lateral acceleration which produces a gravitoinertial stimulus equivalent to lateral head tilt without rotational movement of the head. Results available for two crewmen suggest a decreased amplitude of sinusoidal ocular torsion when measured on the day of landing (R+0) and an increasing amplitude when measured during the week following the mission.

Kenyon RV, and Young LR. M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 5. Postural responses following exposure to weightlessness. *Exp Brain Res* 1986;64:335-346. The four science crewmembers of Spacelab-1 were tested for postural control before and after a 10-day mission in weightlessness. Previous reports have shown changes in astronaut postural behavior following a return to earth's 1-g field. This study was designed to identify changes in EMG latency and amplitudes that might explain the instabilities observed post-flight. Erect posture was tested by having the subject stand on a pneumatically driven posture platform which pitched rapidly and unexpectedly about the ankles causing dorsi- and plantarflexion. Electromyographic (EMG) activity from the tibialis anterior and the gastrocnemius-soleus muscles was measured during eyes open and eyes closed trials. The early (pre 500 ms) EMG response characteristics (latency, amplitude) in response to a disturbance in the posture of the subject were apparently unchanged by the 10 days of weightlessness. However, the late (post 500 ms) response showed higher amplitudes than was found pre-flight. General postural control was quantitatively measured pre- and post-flight by a "sharpened Romberg Rails test". This test showed decrements in standing stability with eyes closed for several days post-flight.

Money KE, Watt DG, and Oman CM. Preflight-and postflight motion sickness testing of the Spacelab 1 crew. In: MOTION SICKNESS: MECHANISMS, PREDICTION, PREVENTION AND TREATMENT. AGARD CP-372 1984;33-1--33-8. The four Spacelab 1 payload crew members, as experimental subjects, were exposed to a variety of motion sickness tests. Contrary to expectation, the crew member who was most susceptible to these tests was the least susceptible to space motion sickness, and the crew member who was most susceptible to space motion sickness was one of the least susceptible to these tests. On the third day after returning from the mission, one of the preflight tests (KC 135) was repeated, and all of the crew members were found to be non-susceptible. Statements of generalities will have to wait for the accumulation of more experimental subjects.

Oman CM. Spacelab experiments on space motion sickness. *Acta Astronautica* 1987;15(1):55-66. Recent research results from ground and flight experiments on motion sickness and space sickness conducted by the Man Vehicle Laboratory are reviewed. New tools developed include a mathematical model for motion sickness, a method for quantitative measurement of skin pallor and blush in ambulatory subjects, and a magnitude estimating technique for ratio scaling of nausea or discomfort. These have been used to experimentally study the time course of skin pallor and subjective symptoms in laboratory motion sickness. In prolonged sickness, subjects become hypersensitive to nauseogenic stimuli. Results of a Spacelab-1 flight experiment are described in which four observers documented the stimulus factors for and the symptoms signs of space sickness. The clinical character of space sickness differs somewhat from acute laboratory motion sickness. However SL-1 findings support the view that space sickness is fundamentally a motion sickness. Symptoms were subjectively alleviated by head movement restriction, maintenance of a familiar orientation with respect to the visual environment, and wedging between or strapping onto surfaces which provided broad contact cues confirming the absence of body motion.

Oman CM, Lichtenberg BK, Money KE, and McCoy RK. M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 4. Space motion sickness: symptoms, stimuli, and predictability. *Exp Brain Res* 1986;64:316-334. Space sickness symptoms were observed by 4 specially trained observers on Spacelab-1. Three reported persistent symptoms, and vomited repeatedly during the first and/or second day of flight. Head movements on all axes were provocative, particularly in pitch and roll. Head acceleration data recorded from 2 symptomatic crewmen showed that after several hours of physical activity in orbit, symptoms appeared and thereafter both crewmen were compelled to limit head movements. Firm body contact with motionless surfaces helped alleviate symptoms. When crewmembers floated into unfamiliar body orientations in the cabin, inherent ambiguities in static visual orientation cues sometimes produced spatial reorientation episodes which were also provocative. Symptoms largely resembled those of other forms of prolonged motion sickness, superimposed upon other symptoms attributable to fluid shift. All 4 eventually used anti-motion sickness drugs. When they did, vomiting frequently was reduced. By the 4th day, symptoms subsided, and head accelerations again increased in magnitude and variability. Sickness intensity in orbit was not predicted by statistically concordant results of 6 acute preflight susceptibility tests. However, results from a longer duration preflight prism goggles test showed an apparent correlation. All subjects were asymptomatic making head movements in parabolic flight 4 days after the mission, but not 1 year later. Overall, results support the view that space sickness is a motion sickness.

Oman CM, Young LR, Watt DGD, Money KE, Lichtenberg BK, Kenyon RV, and Arrott AP. MIT/ Canadian Spacelab experiments on vestibular adaptation and space motion sickness. In: BASIC AND APPLIED ASPECTS OF VESTIBULAR FUNCTION (Eds.) Hwang JC, Daunton NG, and Wilson VJ. Hong Kong: Hong Kong University Press. 1988;183-192. Experiments on sensory-motor adaptation to weightlessness and re-adaptation to 1 g were conducted on Space Shuttle/Spacelab Missions 1 and D-1 by a team of investigators from MIT and Canada. Results from both missions are reviewed in the context of a sensory re-interpretation hypothesis and the conflict theory for motion sickness.

Ross MD. Anatomic evidence for peripheral neural processing in mammalian graviceptors. *Aviat Space Environ Med* 1985;56(4):338-343. Ultrastructural study of utricular and saccular maculas demonstrates that their innervation patterns are complex. There is a clustering of type I and type II hair cells based upon a sharing of afferents, a system of efferent-type beaded fibers that is of intramacular (mostly calyceal) origin, and a plexus-like arrangement of afferents and efferents at many sites in the neuroepithelium. Results

suggest that information concerning linear acceleration is processed peripherally, beginning at the hair cell level, before being sent to the central nervous system. The findings may supply a structural basis for peripheral adaptation to a constant stimulus, and for lateral inhibition to improve signal relative to noise.

Ross MD. Implications of otoconial changes in microgravity. *Physiologist* 1987;30(1,Suppl):S90-S93. Otoconia of maculas of Sprague-Dawley rats (Taconic Farms) flown aboard Spacelab-3 showed no signs of demineralization. Other findings were accumulations of miniature otoconia at the lateral border of utricular patches and a smoothing of surfaces of saccular otoconia. These features were not observed in age- and weight- matched ground controls. Subsequent study showed otoconial asymmetry to be normal in this strain. Further research in space, taking this into account, is clearly required. Findings of ground-based studies would suggest that neural structures of maculas are adaptable to microgravity but might show changes over time. Moreover, maculas have the potential for integration of the sort ascribed to brain and retina, although on a less complex scale. They may act as comparators, and asymmetry may be an important property. Coordinated studies in space and on the ground could lead to new understanding of how maculas function and adapt to new acceleratory environments; and to insights about the functioning of neural tissue in general.

Ross MD. Mammalian macular organization: a model information network. *Adv Oto Rhino Laryngol* 1988;41:142-145. Recent ultrastructural findings in rat maculas have demonstrated that type II hair cells are integrated into the neural circuitry innervating type I cells. This work also showed that three kinds of afferent terminal patterns occur. These are U-, M/U- and M-types, illustrated diagrammatically in figure 1. These and other observations were interpreted to indicate that complex processing of information takes place in the mammalian macula. More recent study of a long series of sections through rat utricular macula supports the notion that type II hair cells link calyces and distribute information. This means that maculas are neural networks morphologically organized for parallel processing of linear acceleratory signals. This paper focuses on macular neural connectivity, especially on networks in pars externa encompassing the three different kinds of nerve/terminal patterns. The evident weighting in directional flow of information and possible physiological implications of the findings are discussed.

Ross MD. Morphological evidence for parallel processing of information in rat macula. *Acta Otolaryngol* 1988;106:213-218. Study of montages, tracings and reconstructions prepared from a series of 570 consecutive ultrathin sections show that rat maculas are morphologically organized for parallel processing of linear acceleratory information. Type II cells of one terminal field distribute information to neighboring terminals as well. The findings are examined in light of physiological data which indicate that macular receptor fields have a preferred directional vector, and are interpreted by analogy to a computer technology known as an information network.

Ross MD. Striated organelles in hair cells of rat inner ear maculas: description and implication for transduction. *Physiologist* 1982;25(6,Suppl):S113-S114. Use of unusual fixation procedures resulted in display of the several striated organelles that are present in hair cells of the inner ear. In the vestibular system these include the striated rootlets of the kinocilia, the striated cuticular plate and its attachments to cell membrane at the zonula adherens, and a striated neck organelle (SNAP) that is present only in Type I hair cells. The possible roles of these organelles in vestibular hair cell transduction are considered here. It is suggested that the kinociliary apparatus serves as a pacemaker for hair cell activity, and that the cuticular

plate functions in part to coordinate kinociliary and stereociliary interactions. The plate also could transmit signals for the apical to the lateral cell membrane and, if contractile, could amplify small signals and produce graded hair cell responses. SNAP is situated at the plasma membrane under the upper end of the calyx nerve ending where it could modulate hair cell electric conductance.

Ross MD, and Bourne C. Interrelated striated elements in vestibular hair cells of the rat. *Science* 1983;220:622-624. Unusual fixation procedures revealed a series of interrelated striated organelles in type I and type II vestibular hair cells of the rat; these organelles seemed to be less well developed in cochlear hair cells. The findings suggest that contractile elements may play a role in sensory transduction in the inner ear, particularly in the vestibular system.

Ross MD, Donovan KM, and Chee O. Otoconial morphology in space-flown rats. *Physiologist* 1985;28(6,Suppl):S219-S220. One question to be answered by spaceflight is whether gravity receptors will show degenerative changes during short- or long- term exposures to microgravity. Weightlessness imposes a new bias against which translational accelerations must be judged. Will the system adjust to this change in bias by undergoing visible alteration or degeneration, or will adaptive processes be more subtle? This ultrastructural study of inner ears obtained from space-flown rats and from age-matched, ground-based controls is our first step in attempting to answer the questions posed. The rats were used primarily for testing the animal holding facility under space-flight conditions rather than for elucidating vestibular responses to microgravity. Nevertheless, it can be stated conclusively that short-term exposure to weightlessness (7 days) does not result in degeneration of macular receptors. It is possible, instead, that a slight increase in otoconial mass occurred. In the utricle, this appeared to be through otoconial neogenesis and in the saccule, by growth of existing otoconia.

Ross MD, Donovan KM, and Rogers C. Peripheral sensory processing in mammalian gravity receptors: observations of ciliary tuft configurations. In: THE VESTIBULAR SYSTEM: NEUROPHYSIOLOGIC AND CLINICAL RESEARCH (Eds.) Graham MD, and Kemink JL. New York: Raven Press. 1987;119-124. Recent findings from our laboratory have provided a morphological basis for peripheral processing of linear acceleratory information by vestibular gravity receptors. Type I and type II cells were organized into functional groups through shared innervations in both the anterior part of the utricular and the inferior portion of the saccular maculae. We now have completed a study of 148 serial sections through the anterior part of the saccular macula. Here, as in the other areas studied, innervation of the type II cells was dependent on the neural circuitry supplying type I cells. During the course of this work, our attention was drawn to the orientations, sizes, and configurations of the ciliary tufts of hair cells comprising functional groupings. Type II hair cell ciliary tufts always consisted of finer and shorter stereocilia than those present on type I cells, and hair cells comprising clusters did not appear to be dynamically polarized in parallel. Ciliary orientations and differences in height have physiological implications for neural processing equal in importance to the neural circuitry underlying type I and type II cell integration. We have therefore begun a coordinated investigation using both transmission electron microscopy (TEM) and scanning electron microscopy (SEM) to determine the dynamic polarizations of clustered cells and to shed some light on the possible meaning of two kinds of hair cells integrated into the same neural circuitry. This paper focuses on preliminary findings of our SEM study.

Ross MD, and Peacor DR. The nature and crystal growth of otoconia in the rat. ***Ann Otol Rhinol Laryngol*** **1975;84(1):22-36.** Several types of otoconia are present in the macular regions of young rats. These include multifaceted, transitional and rounded body forms, some variant otoconia and a few rhombohedrons. The adult form has typically rounded but nonsmooth body surfaces and pointed ends with three planar faces. The multifaceted and transitional otoconia fracture and etch more readily than do the adult type. The differences in properties of the otoconia are considered in the light of known facts concerning inorganic crystal nucleation and growth. This integrated approach indicates that many otoconia originate by seeding of multiple subunits on an organic substrate and develop by the mechanism of parallel growth. The basic structural unit is the rhombohedron. By analogy to inorganic crystals of calcite, it would seem that the typical otoconium grows on the end faces but growth on the side faces is suppressed by some unknown chemical factor. Some otoconia are exceptions, evidently seeding and growing in the pure rhombohedral form. Decalcification of cleaved otoconia shows that organic material is incorporated during growth. The observations are interpreted to indicate that organic substance influences growth and achievement of the adult otoconial form.

Ross MD, Peacor DR, Johnsson LG, and Allard LF. Observations on normal and degenerating human otoconia. ***Ann Otol Rhinol Laryngol*** **1976;85(3):310-326.** Specimens of human otoconia obtained from autopsy material and representing various stages from fetal to advanced old age, were studied by microdissection, scanning electron microscopy, electron microprobe analysis, and x-ray powder diffraction. The typical adult otoconial configuration is a cylindrical, finely serrated body with pointed ends; crystallographically, it corresponds to a single crystal of calcite. Other, less numerous types include joined otoconia, pure rhombohedrons and multifaceted, presumably immature forms. Many otoconia achieve the adult configuration during fetal development. The multifaceted otoconia are most numerous, and the rhombohedrons proliferate, during childhood in the utricle. Crystals from both end organs are virtually identical in composition in the young adult, but saccular otoconia are the larger. In middle and advanced age the otoconia decrease in number, especially in the saccule. Saccular otoconia degenerate progressively in a posteroanterior direction across the macula; they assume a specific, fibrous, hollowed-out appearance, which is not duplicated by either chemical etching or autolysis. Neogenesis and growth of otoconia appear to occur postnatally, with different characteristic growth potentials for those of the saccule and the utricle. Age-related saccular otoconial degeneration appears to involve the organic material, which disappears either before or simultaneously with the mineral substance.

Ross MD, and Pote KG. Some properties of otoconia. ***Phil Trans R Soc Lond*** **1984;B304:445-452.** Otoconia are dynamic mineral deposits present in the gravity receptors of most vertebrates; fishes often have a single large mass called an otolith instead. Otoconia generally have the appearance of single crystals but contain organic and inorganic components, the mineral being almost exclusively a polymorph of calcium carbonate. The two phases are closely interrelated structurally. Ultra-high resolution transmission electron microscopy of rat otoconia showed them to be mosaic biominerals. The crystallites were 50-100 nm in diameter, had some rounded edges, and were highly ordered into laminae. This suggests that crystallite seeding and growth is organic matrix mediated. Crystallite asymmetry may also indicate piezoelectricity. A further finding of similarities in electron beam diffraction patterns obtained from some frog and rat otoconia could mean that the calcite of mammalian units mimics aragonite. A comparative study showed that turtles, which are close to the stem line for mammals, had calcite-type otoconia in the utricle. Alligators, which share a common ancestry with birds, had this type otoconium in all three gravity receptors, although saccular otoconia had a variety of forms. The nature of the mineral is unknown. The biochemical composition of the organic otoconial material is under study, to learn how mineral deposition is regulated.

Proteins of rat otoconial complexes ranged between *ca.* 16500 and over 100 000 Da in molecular mass and were similar in saccular and utricular otoconial complexes. Our new analysis of the amino acid composition of the complexes by high performance liquid chromatography showed the complexes to be high in the acidic and low in the basic amino acids. This is comparable to what has already been reported for other biomineralized materials that contain calcite.

Ross MD, Pote KG, Cloke PL, and Corson C. In vitro $^{45}Ca^{++}$ uptake and exchange by otoconial complexes in high and low K^+/Na^+ fluids. *Physiologist* 1980;23(6,Suppl):S129-S130. Recently, data have been accumulating to indicate that saccular and utricular otoconial complexes of the gravity receptor organs are dynamic and interact constantly with their environment. This study investigates the possibility that the ionic composition of the surrounding fluid influences calcium ion binding and release, and explores the importance of the K^+/Na^+ ratio. Two in vitro methods were developed, the first of which employed artificial endolymph and perilymph while ionically balanced fluids in which only the K^+/Na^+ was altered were used in the second. The ability of rat complexes to take up $^{45}Ca^{++}$ during incubation with these fluids was assessed using liquid scintillation spectrometry. In vitro uptake of $^{45}Ca^{++}$ was greater in fluids with a high K^+/Na^+ ratio than in fluids in which the ratio was low. The ability of the complexes to take up $^{45}Ca^{++}$ appeared to decline with age.

Ross MD, Pote KG, Rarey KE, and Verma LM. Microdisc gel electrophoresis in sodium dodecyl sulfate of organic material from rat otoconial complexes. *Ann NY Acad Sci* 1981;374:808-819.

Ross MD, Rogers CM, and Donovan KM. Innervation patterns in rat saccular macula. *Acta Otolaryngol* 1986;102:75-86. Serial sections through the anterior part of rat saccular macula were reconstructed as montages. Findings are that type II hair cells are integrated into the neural circuitry of type I cells, chiefly by synapses with neighboring calyces and their collaterals; and that complex interactions between afferent- and efferent-type nerve elements take place. Three basic types of nerve/calyx pattern are present: U-type nerves lose their myelin before they enter the macula and have complex calyces with several collaterals; M-type nerves are myelinated up to the calyx, which lacks collaterals; and M/U-type nerves have short, unmyelinated segments proximal to their calyces, which have few collaterals. Both afferent- and efferent-type collaterals spring for calyces, chiefly for those of U-type nerves. Type II cells are presynaptic both to electron-lucent and to vesiculated terminals; some synapses are reciprocal. Electronlucent boutons sometimes are presynaptic to calyces and to type II hair cells; and morphologically afferent-to-afferent kinds of synapses occur in the neuroepithelium. The anatomical findings indicate that complex information processing must occur in mammalian gravity receptors.

Ross MD, and Williams TJ. Otoconial complexes as ion reservoirs in endolymph. *Physiologist* 1979;22(6,Suppl):S63-S64. Otoconia of the gravity receptors and their membranes are complex mineral deposits of calcite crystals and organic substance. $^{45}Ca^{2+}$ uptake and exchange in these complexes and in bone mineral were studied in young adult Wistar rats using microdissection procedures, pooled otoconial samples and the sensitive method of liquid scintillation spectrometry. Intraperitoneal injection of $^{45}Ca^{2+}$ (4 mCi/kgm body weight) resulted in rapid uptake into saccular complexes (15 mins) but uptake into utricular complexes took longer (up to two hrs). Although retention of $^{45}Ca^{2+}$ in saccular complexes was higher throughout the one month experimental period, major uptake had occurred in both complexes by 4 hrs. This time frame is constant with that for entry of the major part of an injected dose of $^{45}Ca^{2+}$ into bone. $^{45}Ca^{2+}$ declined in otoconial

complexes after 4 days but remained high in bone mineral. The results indicate that otoconial complexes are dynamic mineral deposits but function in distinct environments at the two sites. It is suggested here that otoconial complexes not only provide mass to enhance gravity receptor function but also act as ion reservoirs, contributing to the ionic stability of the endolymph.

Salamat MS, Ross MD, and Peacor DR. Otoconial formation in the fetal rat. ***Ann Otol Rhinol Laryngol*** **1980;89(3):229-238.** The development of otoconia in the fetal rat was investigated by scanning and transmission electron microscopy and by x-ray elemental analysis. The transmission electron microscopical results indicate that primitive otoconia are highly organic appearing but are trigonal in cross section, indicating that they already possess a three-fold axis of symmetry and a complement of calcite. These otoconia develop into spindle-shaped units which accrue fibrous, organic material at an angle to their surfaces. Dumbbell-shaped otoconia, with distinct central cores and peripheral zones, result. These otoconia then mature to the adult crystal configuration having a more cylindrical body and pointed ends. The existence of trigonal, spindle- and dumbbell-shaped otoconia was verified by scanning electron microscopy of fresh-frozen material. Tissues prepared for transmission electron microscopy proved (by elemental analysis) to have been decalcified inadvertently, fortuitously revealing the arrangement of the organic material. Subsequent transmission electron microscopy of dumbbell-shaped otoconia not exposed to fluids during embedment showed that calcite deposits mimicked the arrangement of the organic material. X-ray elemental analysis demonstrated that calcium was present in lower quantities in the central core than peripherally. Findings are interpreted to indicate that organic material is essential to otoconial seeding and directs otoconial growth.

Shelhamer M, Marino LA, Young LR, Arrott AP, and Wiseman JJ. Normative study of Spacelab preflight/postflight vestibular test battery. ***Aviat Space Environ Med*** **1987;58(9,Suppl):A236-A239.** A study was designed to establish baseline normative responses to the MIT/Canadian Spacelab vestibular test battery. Three tests used a linear acceleration sled to measure otolith function: 1) perception of linear motion (threshold determination); 2) compensatory eye movements (linear VOR); 3) closed-loop nulling, in which the blindfolded subject nulls his velocity with a joystick under the influences of a pseudorandom sled disturbance. Rotational VOR was measured at 0.3 and 0.8 Hz in the dark and the light. Static visual-vestibular interaction was tested with a standard rod and frame apparatus, while dynamic interaction was assessed by susceptibility to roll vection induced by a rotating peripheral visual field. Two examples are presented of how results from this study can aid in the interpretation of data from preflight/postflight testing of Space Shuttle/Spacelab crews on these same experiments.

Watt DGD, Money KE, Bondar RL, Thirsk RB, Garneau M, and Scully-Power P. Canadian medical experiments on shuttle flight 41-G. ***Can Aeronaut Space J*** **1985;31(3):215-226.** During the 41-G mission, two payload specialist astronauts took part in six Canadian medical experiments designed to measure how the human nervous system adapts to weightlessness, and how this might contribute to space motion sickness. Similar tests conducted pre-flight provided base-line data, and post-flight experiments examined re-adaptation to the ground. No changes were detected in the vestibulo-ocular reflex during this 8-day mission. Pronounced proprioceptive illusions were experienced, especially immediately post-flight. Tactile acuity was normal in the fingers and toes, but the ability to judge limb position was degraded. Estimates of the locations of familiar targets were grossly distorted in the absence of vision. There were no differences in taste thresholds or olfaction. Despite pre-flight tests showing unusual susceptibility to motion sickness, the Canadian payload specialist turned out to be less susceptible than normal on-orbit. Re-adaptation to the normal gravity environment occurred within the first day after landing.

Watt DGD, Money KE, and Tomi LM. M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 3. Effects of prolonged weightlessness on a human otolith-spinal reflex. ***Exp Brain Res*** **1986;64:308-315.** Reflex responses that depend on human otolith organ sensitivity were measured before, during and after a 10 day space flight. Otolith-spinal reflexes were elicited by means of sudden, unexpected falls. In weightlessness, "falls" were achieved using elastic cords running from a torso harness to the floor. Electromyographic (EMG) activity was recorded from gastrocnemius-soleus. The EMG response occurring in the first 100-120 ms of a fall, considered to be predominantly otolith-spinal in origin, decreased in amplitude immediately upon entering weightlessness, and continued to decline throughout the flight, especially during the first two mission days. The response returned to normal before the first post-flight testing session. The results suggest that information coming from the otolith organs is gradually ignored by the nervous system during prolonged space flight, although the possibility that otolith-spinal reflexes are decreased independent of other otolith output pathways cannot be ruled out.

Young LR. Tilted astronauts reveal the brain's balancing act. ***New Sci*** **1984.**

Young LR, Oman CM, Watt DGD, Money KE, and Lichtenberg BK. Spatial orientation in weightlessness and readaptation to Earth's gravity. ***Science*** **1984;225:205-208.** Unusual vestibular responses to head movements in weightlessness may produce spatial orientation illusions and symptoms of space motion sickness. An integrated set of experiments was performed during Spacelab 1, as well as before and after the flight, to evaluate responses mediated by the otolith organs and semicircular canals. A variety of measurements were used, including eye movements, postural control, perception of orientation, and susceptibility to space sickness.

Young LR, Shelhamer M, and Modestino S. M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 2. Visual vestibular tilt interaction in weightlessness. ***Exp Brain Res*** **1986;64:299-307.** Adaptation to weightlessness includes the substitution of other sensory signals for the no longer appropriate graviceptor information concerning static spatial orientation. Visual-vestibular interaction producing roll circularvection was studied in weightlessness to assess the influence of otolith cues on spatial orientation. Preliminary results from four subjects tested on Spacelab-1 indicate that visual orientation effects were stronger in weightlessness than pre-flight. The rod and frame test of visual field dependence showed a weak post-flight increase in visual influence. Localized tactile cues applied to the feet in space reduced subjective vection strength.

CARDIOVASCULAR AND CARDIOPULMONARY

Arieli R, Boutellier U, and Farhi LE. Effect of water immersion on cardiopulmonary physiology at high gravity (+Gz). *J Appl Physiol* 1986;61(5):1686-1692. We compared the cardiopulmonary physiology of eight subjects exposed to 1, 2, and 3 G_z during immersion (35°C) to the heart level with control dry rides. Immersion should almost cancel the effects of gravity on systemic circulation and should leave the lung alone to gravitational influence. During steady-state breathing we measured ventilation, O_2 consumption (VO_2), CO_2 production, end-tidal PCO_2 ($PACO_2$), and heart frequency (f_H). Using CO_2 rebreathing techniques, we measured cardiac output, functional residual capacity, equivalent lung tissue volume, and mixed venous O_2 content, and we calculated arterial PCO_2 ($PACO_2$). As G_z increased, ventilation, f_H, and VO_2 rose markedly, and $PACO_2$ and $PaCO_2$ decreased greatly in dry ride, but during immersion these variables changed very little in the same direction. Functional residual capacity was lower during immersion and decreased both the dry and immersed states as G_z increased, probably reflecting closure effects. Cardiac output decreased as G_z increased in dry rides and was elevated and unaffected by G_z during immersion. We conclude that most of the changes we observed during acceleration are due to the effect on the systemic circulation, rather than to the effect on the lung itself.

Arieli R, and Farhi LE. Gas exchange in tidally ventilated and non-steadily perfused lung model. *Respir Physiol* 1985;60:295-309. We studied the effect of cyclic lung perfusion - fast cycle in synchrony with heart beats and slow cycle in synchrony with ventilation - on gas exchange in a lung model. There was almost no effect in the fast cycle. In a homogenous single-lung unit, arterial PO_2 increased, and the $(A\text{-}a)DO_2$ decreased (by approximately 0.5Torr), as the amplitude of the slow cyclic lung perfusion (TIP) increased. The calculated $(A\text{-}a)DO_2$ and $(a\text{-}A)DCO_2$ were negative. Maximal Pa_{O2} was found when peak lung perfusion was delayed with respect to ventilation by 0.2 of a cycle. In a non-homogeneous nine-unit lung, cyclic lung perfusion caused an increase in Pa_{O2} and a decrease in $(A\text{-}a)DO_2$ by 2 Torr as compared to steady perfusion. No apparent negative $(A\text{-}a)DO_2$ was found, but apparent negative $(a\text{-}A)DCO_2$ was calculated at no pulmonary shunt and also with 5% shunt. The correlation of cyclic lung perfusion to the reduced $(A\text{-}a)DO_2$ in dense-gas breathing - where large swings of pleural pressure are expected - and its effect on the diffusion capacity of the lung are discussed. Non-steady perfusion of the lung as caused by ventilatory movements expanded our understanding of gas exchange and shed some light on a few controversial experimental findings, such as the negative $(a\text{-}A)DCO_2$, the decreased $(A\text{-}a)DO_2$ while breathing dense gas, and the effects of gas density on diffusion capacity of the lung.

Arieli R, and Farhi LE. Gravity-induced hyperventilation is caused by a reduced brain perfusion. *Respir Physiol* 1987;69:237-244. The suggestion that hyperventilation caused by increased gravity is mediated by a decrease in brain perfusion has led us to propose a mathematical model based on: (1) the CO_2 balance equation for the respiratory center (RC), and (2) the relationship between RC blood flow (QRC), foot-to-head acceleration (G_z) and $PRCCO_2$, namely, $QRC=[1 - a(G_z - 1)](b\ PRCco_2 + c)$, where the coefficients a, b and c can be calculated from data in the literature. QRC is significantly affected by $+G_z$ only at high $PaCO_2$. The model can be used to calculate oxygen pressure in the RC; the numbers so obtained are in good agreement with measurements of jugular vein PO_2 obtained by others.

Blomqvist CG. Orthostatic hypotension. In: CARDIOLOGY (Eds.) Parmley WW, and Chatterjee K. Philadelphia: J. B. Lippincott, 1990;1-12. Orthostatic hypotension is a common and sometimes disabling condition. Its pathophysiology has been studied extensively. Likely causes include many different defects that singly or in combination affect major mechanisms controlling blood flow, vascular resistance, arterial pressure, and intravascular volume. The control systems are complex, and their interactions are poorly understood. As a consequence, obvious and straight-forward therapeutic approaches often prove

ineffective, but seemingly paradoxical measures are sometimes helpful. These characteristics combine to make orthostatic hypotension a challenging topic. This review deals mainly with orthostatic hypotension occurring in the absence of structural neurological lesions.

Blomqvist CG. Orthostatic hypotension. ***Hypertension*** **1986;8(8):722-731.**

Blomqvist CG, Gaffney FA, and Nixon JV. Cardiovascular responses to head-down tilt in young and middle-aged men. ***Physiologist*** **1983;26(6,Suppl):S81-S82.**

Blomqvist CG, Nixon JV, Johnson RL, and Mitchell JH. Early cardiovascular adaptation to zero gravity simulated by head-down tilt. ***Acta Astronautica*** **1980;7(4/5):543-553.** The early cardiovascular adaptation to zero gravity, simulated by head-down tilt at 5°, was studied in a series of 10 normal young men. The validity of the model was confirmed by comparing the results with data from Apollo and Skylab flights. Tilt produced a significant central fluid shift with a transient increase in central venous pressure, later followed by an increase in left ventricular size without changes in cardiac output, arterial pressure, or contractile state. The hemodynamic changes were transient with a nearly complete return to the control state within 6 hr. The adaptation included a diuresis and a decrease in blood volume, associated with ADH, renin and aldosterone inhibition.

Boutellier URS, Arieli R, and Farhi LE. Ventilation and CO_2 response during +Gz acceleration. ***Respir Physiol*** **1985;62:141-151.** During foot-to-head acceleration ($+G_z$) ventilation increases despite a drop in alveolar P_{CO_2}. In order to investigate the underlying mechanisms, we measured ventilation (VE), V_{O_2}, V_{CO_2} and PA_{CO_2}, cardiac output (Q) and mixed venous CO_2 concentration (C_{VCO_2}) using non-invasive techniques in 5 subjects breathing either air or a gas mixture containing 5% CO_2 at +1, +2 and +3 G_z in a human centrifuge. Arterial P_{CO_2} was calculated from Fick's equation, using C_{VCO_2}, Q and V_{CO_2}. VE increased from 8.7 to 18.0 L/min during air breathing and from 19.6 to 36.9 L/min during CO_2 breathing at +1 and +3 G_z, respectively. The corresponding values for PA_{CO_2} are 37.9 vs 26.9 Torr and 47.8 vs 46.4 Torr. Q dropped from 5.9 to 4.8 L/min during air breathing and remained the same during CO_2 breathing (6.7 vs 6.5 L/min). As the decrease of Pa_{CO_2} almost paralleled that of PA_{CO_2}, the arterio-alveolar CO_2 difference increased only slightly. The CO_2 response curve shifts gradually to the left with an increase in $+G_z$, a fact that does not support the hypothesis that foot-to-head acceleration increases CO_2 sensitivity.

Boutellier URS, and Farhi LE. Influence of breathing frequency and tidal volume on cardiac output. ***Respir Physiol*** **1986;66:123-133.** The aim of our experiment was to investigate the influence of increasing either breathing frequency or tidal volume on cardiac output (Q), in normocapnia. We measured Q with a CO_2 rebreathing method in 6 men and 6 women in the sitting and the supine position, imposing different breathing patterns: in one set of experiments tidal volume was kept constant a 1 L while breathing frequency was randomly changed between 20, 30 and 40 breaths/min; in another breathing frequency was kept constant at 30 breaths/min while tidal volume was randomly altered between 1, 1.5 and 2L. Switching from open circuit breathing to rebreathing (for measurement of Q) required no change in breathing pattern. From the beginning, CO_2 was added to the inspired gas to maintain end-tidal F_{CO_2} 0.054, so as to obtain steady state conditions throughout the measurements. Q rose significantly when tidal volume was increased (938 ml/L rise in tidal volume when sitting, and 743 ml/L when supine). Breathing frequency had an insignificant effect (213 ml/10 breaths frequency increase when sitting and 142 ml/10 breaths when supine). The greater

influence of ventilation on Q when sitting than when supine is best explained by the fact that in the latter position venous return is already high. There are no demonstrable differences in this effect between males and females.

Boutellier URS, and Farhi LE. A fundamental problem in determining functional residual capacity or residual volume. *J Appl Physiol* 1986;60(5):1810-1813. To measure a lung volume that is not directly accessible, one often follows dilution of a single-gas tracer, present initially only in the lung or in a rebreathing bag. The final volume available to the tracer is assumed to be the sum of the two initial components. Since O_2 is taken up and CO_2 is eliminated during the few breaths required for mixing, the total volume changes. The error in lung volume due to this volume change can exceed 10%. In this paper we 1) present theoretical and experimental data to demonstrate the effect of CO_2 and O_2 exchange, 2) introduce a general equation, based on N_2 and Ar, which allows one to circumvent the problems created by these fluxes, and 3) show the pitfall of the back-extrapolation approach for a single tracer.

Buckey JC, Beattie JM, Gaffney FA, Nixon JV, and Blomqvist CG. Simplified right ventricular volume algorithm using one digitized view and transducer tilt angle. *Comput Cardiol* 1984;399-402. We recently described a technique for determining in-vitro right ventricular volume from multiple two-dimensional echocardiographic views taken at sequential angles. The product of sectional area and center of mass for each view (U) is integrated over the angle of tilt of the transducer to give volume. We now note that the plot of U vs. angle is almost triangular when the echoes are taken from the short axis position. The maximal U value (the vertex of the triangle) and the total angle span (the base) are then used in the equation (maximal U x total angle span)/2 to calculate volume. This new approximation provides an excellent correlation with actual volumes. We conclude that the triangular approximation provides accurate in-vitro estimates of right ventricular volume in normal human hearts.

Buckey JC, Beattie JM, Nixon JV, Gaffney FA, and Blomqvist CG. Right and left ventricular volumes in vitro by a new nongeometric method. *Am J Cardiac Imaging* 1987;1(3):227-233. We present an evaluation of a new nongeometric technique for calculating right and left ventricular volumes. This method calculates ventricular chamber volumes from multiple cross-sectional echocardiographic views taken from a single point as the echo beam is tilted progressively through the ventricle. Right and left ventricular volumes are calculated from both the approximate short axis and approximate apical position on 20 in vitro human hearts and compared with the actual chamber volumes. The results for both ventricles from both positions are excellent. Correlation coefficients are >0.95 for all positions; the standard errors are in the range of 5 to 7 mL and the slopes and intercepts for the regression lines are not significantly different from 1 and 0, respectively (except for the left ventricular short-axis intercept). For all positions, approximately 6 to 8 views are needed for peak accuracy (7.5° to 10° separation). This approach offers several advantages. No geometric assumptions about ventricular shape are made. All images are acquired from a single point (or window), and the digitized points can be used to make a three-dimensional reconstruction of the ventricle. Also, during the calculations a volume distribution curve for the ventricle is produced. The shape of this curve can be characteristic for certain situations (ie, right ventricle, short axis) and can be used to make new simple equations for calculating volume. We conclude that this is an accurate nongeometric method for determining both right and left ventricular volumes in vitro.

Buckey JC, Goble RL, and Blomqvist CG. A new device for continuous ambulatory central venous pressure measurement. *Med Instrum* 1987;21(4):238-243. We have developed a device for continuous direct measurement of human central venous pressure (CVP) during space flight. Normal resting CVP is typically in the range of 5-10 mm Hg; in zero gravity, the expected changes are ± 5 mm Hg or less. A 1 mmHg change in CVP can represent a substantial intravascular fluid shift. The device is small, battery powered, and designed to run for a least 24 hours. Pressure is measured in a saline solution-filled catheter inserted into a central vein. The transducer is placed in the axilla at the level of the catheter tip to offset hydrostatic gradients. A pump and an electronic system mount on the leg. This assembly provides a slow, continuous infusion of heparinized saline solution to maintain the patency of the catheter. The electronic system generates a digital display in mm Hg, an analog output, and a visible and audible alarm for excessive pressure. An air-filled syringe allows for a two-point calibration (zero and a positive pressure generated by measured compression of a known gas volume). A two-failure tolerant system minimizes electric shock hazards. Two latex diaphragms separate the saline solution from the transducer surface, and the electronic system and pump chamber are in separate enclosures. A clear polycarbonate case allows bubbles to be seen. The unit has been tested for pump function, temperature stability, drift, and accuracy. We conclude that this approach provides a unit with sufficient stability, accuracy, and temperature insensitivity for measuring ambulatory CVP for up to 28 hours. The design may be suitable for ambulatory measurement of other intravascular and intracardiac pressures.

Buckey JC, Peshock RM, and Blomqvist CG. Deep venous contribution to hydrostatic blood volume change in the human leg. *Am J Cardiol* 1988;62:449-453. The causes of orthostatic intolerance following prolonged bed rest, head-down tilt or exposure to zero gravity are not completely understood. One possible contributing mechanism is increased venous compliance and peripheral venous pooling. The present study attempted to determine what proportion of the increased calf volume during progressive venous occlusion is due to deep venous filling. Deep veins in the leg have little sympathetic innervation and scant vascular smooth muscle, so their compliance may be determined primarily by the surrounding skeletal muscle. If deep veins make a large contribution to total leg venous compliance, then disuse-related changes in skeletal muscle mass and tone could increase leg compliance and lead to decreased orthostatic tolerance. The increase in deep venous volume during progressive venous occlusion at the knee was measured in 6 normal subjects using calf cross-sectional images obtained with magnetic resonance imaging. Conventional plethysmography was used simultaneously to give an independent second measurement of leg volume and monitor the time course of the volume changes. Most of the volume change at all occlusion levels (20, 40, 60, 80 and 100 mm Hg) could be attributed to deep venous filling (90.2% at 40 mm Hg and 50.6% at 100 mm Hg). It is concluded that a large fraction of the calf volume change during venous occlusion is attributable to filling of the deep venous spaces. This finding supports theories postulating an important role for physiological mechanisms controlling skeletal muscle tone during orthostatic stress.

Buckey JC, Sweeney FM, Kim LT, Beattie JM, Nixon JV, Gaffney FA, and Blomqvist CG. Stroke volume in-vivo using multiple 2D echo views from one echo window. *Comput Cardiol* 1985;293-296. We recently validated in-vitro a new mathematical approach to echocardiographic volume calculation. With this method all echo views are acquired from one point as the transducer is tilted. The angle of tilt of the transducer must be measured. A tilt frame was designed for use in-vivo that does not interfere with imaging. The method was then tested in-vivo by comparing echocardiographic stroke volume with stroke volume determined by the acetylene rebreathing technique. Normal subjects were studied with either lower body negative pressure or continuous isoproterenol infusion. The regression line was Echo SV=-19.7 + 1.2*Acet SV, with R=0.80, SEE=17.1, MPE=17%. We conclude that this is an accurate, non-geometric method for ventricular volume calculation.

Buckey JC, Watenpaugh DE, Kim LT, Smith ML, Gaffney FA, and Blomqvist CG. Initial experience with a new plethysmograph for zero-g use. ***Physiologist*** **1985;28(6,Suppl):S145-S146.** We developed and tested a system for venous occlusion plethysmography (SVOP) suited for use in Spacelab. This unit measures changes in limb circumference by using an optical shaft encoder connected to a band encircling the limb. Circumference changes are converted to digital pulses and displayed. Flow is then calculated in ml/min/100 ml. This instrument was compared to mercury-in-silastic (Whitney) gauges at rest and with hyperemia after 3 min of arterial occlusion. Overall the correlation coefficient was 0.95, SVOP=0.90 x Whitney + 0.44; at rest alone the correlation coefficient was 0.89, SVOP=0.62 x Whitney + 0.75. We conclude the SVOP produces good flow data, needs no calibration, and contains no toxic materials making it suitable for zero-G use.

Convertino VA, Doerr DF, Eckberg DL, Fritsch JM, and Vernikos-Danellis J. Carotid baroreflex response following 30 days exposure to simulated microgravity. ***Physiologist*** **1989;32(1,Suppl):S67-S68.**

Fritsch JM, Rea RF, and Eckberg DL. Carotid baroreflex resetting during drug-induced arterial pressure changes in humans. ***Am J Physiol*** **1989;256(25):R549-R553.** We studied human baroreflex resetting during 25 min of drug-induced arterial pressure changes in 10 healthy volunteers. Average (± SE) base-line systolic pressure of 113 ± 4 fell to 102 ± 3 during nitroprusside infusions and rose to 135 ± 6 mmHg during phenylephrine infusions. Average base-line R-R intervals of 932 ± 37 shortened to 820 ± 39 during nitroprusside infusions and lengthened to 1,251 ± 61 ms during phenylephrine infusions. Carotid baroreceptor-cardiac reflex responses were evaluated with a complex series of neck chamber pressure changes, and R-R intervals were plotted as functions of carotid distending pressure. Baroreceptor-cardiac reflex relations shifted on both R-R interval and arterial pressure axes during drug infusions, but there was no significant change of the maximum slope or range of R-R interval responses. The position of baseline R-R intervals on the reflex relation (operational point) changed significantly. Resting R-R intervals were closer to threshold during pressure reductions and closer to saturation for baroreceptor-cardiac responses during pressure elevations. These results document short-term partial resetting of human baroreceptor-cardiac reflex responses as early as 25 minutes after the onset of arterial pressure changes.

Gaffney FA, Bastian BC, Thal ER, Atkins JM, and Blomqvist CG. Passive leg raising does not produce a significant or sustained autotranfusion effect. ***J Trauma*** **1982;22(3):190-193.** Passive leg raising is widely used to treat hypotension associated with hypovolemia. Presumably gravity causes a central translocation of leg venous blood and an increase in filling pressure, cardiac output, and arterial pressure. Ten healthy volunteers, 25 to 35 years old, had measurements of heart rate, blood pressure, and cardiac output in the supine position after 20 sec and 7 min of 60° passive leg elevation. The protocol was performed 3 and 45 min after the subjects changed from an ambulatory upright to a supine position. Stroke volume and cardiac output increased transiently (8-10%) when the legs were raised after 3 min rest in the supine position. By 7 min of leg elevation, these beneficial effects disappeared. After 45 min supine, leg raising had no effect on stroke volume or cardiac output but increased blood pressure (4 mm Hg) by increasing peripheral resistance (15%). Thus, leg raising, like application of the MAST trousers, fails to produce any sustained increase in cardiac output or stroke volume. Small venous leg volumes and time-dependent changes in the distribution of venous volume and compliance may explain the absence of any sustained 'autotransfusion' effect.

Gaffney FA, Lane LB, Pettinger W, and Blomqvist CG. Effects of long-term clonidine administration on the hemodynamic and neuroendocrine postural responses of patients with dysautonomia. *Chest* 1983;83S(Feb,Suppl):S436-S438. Patients with mitral valve prolapse syndrome (MVPS), vasoregulatory asthenia, and poor postural adjustment often have orthostatic intolerance characterized by tachycardia and a narrow pulse pressure on standing. Autonomic dysfunction is thought to play an important role. Increased α-adrenergic activity has been shown in MVPS patients with orthostatic intolerance. We measured hemodynamic and neuroendocrine responses to long-term oral clonidine therapy in eight women, aged 36 ± 1.8 years (27 to 44 years). None had responded favorably to ß-blockers. Heart rate, blood pressure, oxygen consumption, cardiac output, and plasma norepinephrine levels were measured in both supine and standing positions, before and after one to four weeks of clonidine (0.3 to 0.4 mg daily). Clonidine reduced standing plasma norepinephrine levels, total peripheral resistance, and diastolic blood pressure; a smaller decrease in cardiac output on standing was noted. Plasma volumes increased 12 percent. Mild reductions in plasma catecholamines and total peripheral resistance are associated with fewer, not more, orthostatic symptoms in this group of patients. "Placebo" or mild sedative effects may explain part of the response to clonidine, but the hemodynamic and neuroendocrine data suggest that decreased α-adrenergic hyperactivity may also be important.

Gaffney FA, Nixon JV, Karlsson ES, Campbell W, Dowdey ABC, and Blomqvist CG. Cardiovascular deconditioning produced by 20 hours of bedrest with head-down tilt (-5°) in middle-aged healthy men. *Am J Cardiol* 1985;56:634-638. Cardiovascular deconditioning after prolonged bedrest has been attributed to inactivity. To examine the role of the altered distribution of body fluid, 5 healthy men, aged 41 to 48 years, were studied before, during and after a 20-hour period of bedrest with head-down tilt (-5°). This intervention produces a marked central shift of intravascular and interstitial fluid, but the short duration minimizes the effects of inactivity. Central venous pressure, cardiac output and stroke volume all increased significantly ($p<0.05$) from supine baseline mean values; central venous pressure from 8.6 to 12.6 cm H_2O, cardiac output from 6.9 to 7.9 liters/min, and stroke volume from 104 to 113 ml after 15 minutes of tilt, but all values returned to baseline within 20 hours. Supine central venous pressure after tilt was 7.4 cm H_20, cardiac output 5.7 liters/min and stroke volume 84 ml. Blood volume decreased 0.51 liters. After tilt, orthostatic stress produces a higher heart rate (90 ± 18 vs 68 ± 12 beats/min). Maximal oxygen consumption decreased (2.36 ± 0.41 vs 2.62 ± 0.48 liters/min), mainly owing to reduced stroke volume (87 ± 22 vs 107 ± 18 ml, $p<0.05$). Thus, tilt produced a transient increase in central venous pressure, stroke volume and cardiac output, but supine mean values were below baseline levels after 20 hours. The post-tilt state was qualitatively and quantitatively similar to that seen after 2 to 3 weeks of bedrest or several days of spaceflight. These results are also similar to those from a previously studied group of ten 20- to 30-year-old normal men. However, the increase in central venous pressure tended to be larger and of longer duration in the middle-aged group. Furthermore, in the young men the initial increase in stroke volume produced relative bradycardia, with no change in cardiac output and arterial pressure. Cardiac output increased in the middle-aged group, but arterial pressure was controlled by vasodilatation. Heart rate did not change. The results support the concept that cardiovascular deconditioning after bedrest is primarily an adaptation to a postural fluid shift rather than to inactivity. Age-related differences in hemodynamic responses to central fluid shifts appear to be present.

Gaffney FA, Thal ER, Taylor WF, Bastian BC, Weigelt JA, Atkins JM, and Blomqvist CG. Hemodynamic effects of medical anti-shock trousers (MAST garment). *J Trauma* 1981;21(11):931-937. Despite widespread use of the Medical Anti-Shock Trousers (MAST) little is known about the exact mechanism by which they increase arterial pressure. It is assumed that an autotransfusion occurs. To examine this question, blood pressure, heart rate, forearm blood flow, cardiac output, and stroke volume were measured in ten healthy adults, supine and during 60° headup tilt with MAST garment pressures of 40 and

100 mm Hg. Supine, the garment produced no *net* 'autotransfusion,' but raised blood pressure (27%) by increasing peripheral resistance (48%) with decreased stroke volume and cardiac output (18%). During headup tilt without the MAST device, venous pooling in the legs decreased stroke volume (52%), cardiac output (30%), and increased total peripheral resistance (40%). Application of the garment during tilt shifted this blood centrally, producing increased stroke volume (14%). In supine normovolemic subjects, the garment raised pressure almost exclusively by increased systemic afterload. Forearm vascular resistance did not change and the increased pressure augmented flow to the arm, i.e., to noncompressed tissue. With increased venous pooling during tilt, the MAST garment acted as a 'G-suit' and caused a central shift of blood volume. These findings could explain: 1) why fluid replacement is not always adequate to maintain pressure when deflating the trousers; 2) why the trousers should not be used if one wished to avoid increasing afterload (e.g., certain patients with acute myocardial infarction). We conclude that the MAST garment acts as a local, effective, nonpharmacologic vasoconstrictor and should be used when such an effect is clinically appropriate.

Guy HJ, Gaines RA, Hill PM, Wagner PD, and West JB. Computerized, noninvasive tests of lung function. A flexible approach using mass spectrometry. ***Am Rev Respir Dis*** **1976;113:737-744.** The design, operation, and some applications of a computerized pulmonary function testing system built around a mass spectrometer are described. The test sequence, performed in 10 to 20 min, includes spirometry, a single-breath N_2 washout, and measurement of the diffusing capacity of the lung for CO. Secondary tests, an integral part of the sequence, include rebreathing estimates of lung volume and cardiac output, and a breath-by-breath analysis of over-all gas exchange. These secondary tests lead to computer modeling of a one-compartment lung closely matched to the subject's lungs. Differences between alveolar plateau slopes in the model and real lung provide information about the degree of ventilation-perfusion mismatch in the subject. It is expected that the combination of tests will be useful in the early detection of lung disease.

Kasting GA, Eckberg DL, Fritsch JM, and Birkett CL. Continuous resetting of the human carotid baroreceptor-cardiac reflex. ***Am J Physiol*** **1987;252:R732-R736.** Although human baroreflex responses have been studied during night as well as day, there has been no attempt to distinguish circadian changes of baroreflex function from those related to sleep. We measured carotid baroreceptor-cardiac reflex responses serially during a 24-h period in 11 normotensive volunteers who were awake and cooperative during testing. We applied sequences of ramped R-wave triggered neck chamber pressure changes from +40 to -65 mmHg, during held expiration, at 3-h intervals. Subjects maintained their usual sleep-wake cycles but were awakened for three 30-min periods for night testing. There was no systematic change of baroreflex slope during the 24-h period. There were, however, parallel shifts of the entire sigmoid baroreceptor-cardiac reflex response relation along its R-R interval and arterial pressure axes associated with small, but significant, circadian changes of baseline R-R intervals and arterial pressures. Thus, although our data do not point toward major circadian variability of baroreflex responsiveness, they provide evidence for an ongoing process of human baroreflex resetting.

Michels DB, and West JB. Distribution of pulmonary ventilation and perfusion during short periods of weightlessness. ***J Appl Physiol*** **1978;45(6):987-998.** Information on the distributions of pulmonary ventilation and perfusion was obtained from four subjects on board a Learjet during 112 weightless periods lasting up to 27 s each. Zero gravity (G) was obtained during all or part of each test by varying the aircraft flight profile. Single-breath N_2 washouts were performed with the test inspiration containing an initial bolus of argon at residual volume (RV). When the test inspiration was at 0 G, and the washout at 0 G or greater, the terminal rises and the cardiogenic oscillations in both N_2 and argon were small and often absent. If instead the test inspiration was at 1 G with the washout at 0 G, the terminal rises were again small or absent but the

cardiogenic oscillations remained. The terminal rise and the cardiogenic oscillations for N_2, but not argon, were also nearly eliminated by performing just the preliminary exhalation to RV at 0 G with the test inspiration and washout following at 1 G. Alveolar plateaus for N_2 sloped upward at 0 G apparently due to nontopographical inequalities of ventilation. In further tests during air breathing, recordings were made of expired partial pressure of oxygen (PO_2) and carbon dioxide (PCO_2) following a brief hyperventilation and a 15-s breath hold. These recordings revealed marked cardiogenic oscillations in PO_2 and PCO_2 at 1 G that were enhanced at 2 G but almost eliminated at 0 G. The results suggest that virtually all the topographical inequality of ventilation, blood flow, and lung volume seen under 1-G conditions are abolished during short periods of 0 G.

Nichol GM, Michels DB, and Guy HJB. Phase V of the single-breath washout test. *J Appl Physiol* 1982;52(1):34-43. A downward-deflecting phase V is often seen following the phase IV terminal rise in the single-breath N_2 washout test (SB N_2). This phase V was studied in eight normal nonsmoking subjects aged 27-41, using both the SB N_2 test and single-breath washouts of boluses of inert tracer gas slowly inhaled from residual volume (RV). All of the subjects showed a distinct phase V in both tests. Expiratory flow rates between 0.1 and 2.0 l/s were used; at each flow rate phase V appeared shortly after expiration became flow limited. Thus the volume above RV at which phase V began increased with increasing expiratory flow rate. The difference between the exhaled volumes at which flow became limited and phase V appeared was shown to be approximately equal to the anatomic dead space. This behavior is predicted by a model of lung emptying in a gravitational field. As expiration proceeds, flow limitation occurs first in the (tracer-poor) lower lung regions and then progresses toward the (tracer-rich) upper lung regions causing phase IV. When all lung regions have finally become flow limited, the amount of flow from the upper regions decreases relative to that of the lower regions, thereby causing phase V.

Nixon JV, Murray RG, Bryant C, Johnson RL, Mitchell JH, Holland OB, Gomez-Sanchez C, Vergne-Marini P, and Blomqvist CG. Early cardiovascular adaptation to simulated zero gravity. *J Appl Physiol* 1979;46(3):541-548. Physiological responses characterizing the early adaptation to weightlessness were studied in five normal men. Supplementary data on central venous pressure (CVP) were obtained in three additional subjects. Zero gravity was simulated by a 24-h period of head-down tilt at 5°. Tilt produced a central fluid shift. Orthostatic tolerance and exercise capacity were reduced posttilt. These changes were similar to those observed during and after space flight and support the validity of the experimental model. CVP increased transiently from 5.6 to a peak of 8.5 cmH_2O ($P<0.02$). Control levels for CVP were approached at 90 min, at that time the echocardiographic left ventricular end-diastolic diameter reached a maximum (4.7 cm, control 3.9 cm, $P<0.05$). There were no changes in arterial pressure, cardiac output, or left ventricular contractile state. Urine flow was 1.98 $ml \cdot min^{-1}$ during the initial 8 h compared to 1.36 during the final 16 h ($P<0.05$). Blood volume decreased by 0.5 liter ($P<0.05$). Plasma renin activity, aldosterone, and antidiuretic hormone were depressed initially but returned to base line within 24 h. Plasma electrolytes remained unchanged. The results suggest that hemodynamic adaptation occurs rapidly and is essentially accomplished by 6 h. Adaptation includes a diuresis and reduction in blood volume.

Nixon JV, Saffer SI, Lipscomb K, and Blomqvist CG. Three-dimensional echoventriculography. *Am Heart J* 1983;106(3):435-443. A method of generating a three-dimensional image of the human left ventricle by computer techniques is described. The volume of each image was estimated by a modification of Simpson's rule. The method was applied to nine suitable patients and estimations of end-diastolic and end-systolic volumes were compared to volumes determined by cineangiography. Significant linear correlation coefficients of 0.95 and 0.94 were obtained for end-diastolic and end-systolic volumes,

respectively. The standard errors of estimate were 9 ml for end-diatsolic volumes and 7 ml for end-systolic volumes. The value of this methodology lies in the ability to estimate left ventricular volumes with accuracy, using an imaging technique of little inconvenience and no risk to the patient and computer hardware that is readily available at most clinical institutions.

Parra B, Buckey J, DeGraff D, Gaffney FA, and Blomqvist CG. Echocardiographic measurements of left ventricular mass by a non-geometric method. ***Aviat Space Environ Med*** **1987;58(9,Suppl):A64-A68.** The accuracy of a new non-geometric method for calculating left ventricular myocardial volumes from 2-D echocardiographic images was assessed *in vitro* using 20 formalin-fixed normal human hearts. Serial oblique short axis images were acquired from one point at 5° intervals, for a total of 10 to 12 cross sections. Echocardiographic myocardial volumes were calculated as the difference between the volumes defined by the epi- and endo-cardial surfaces. Actual myocardial volumes were determined by water displacement. Volumes ranged from 80 to 174 ml (mean 130.8 ml). Linear regression analysis demonstrated excellent agreement between echocardiographic (X) and direct measurements (Y) i.e., $y = 0.98 x + 4.3$ ml; $r = 0.94$; SEE = 8.4; $p = 0.001$. Comparison of 10 duplicate measurements by two independent observers yielded an r of 0.96. These *in vitro* results suggest that with this technique, quantitative analysis of a limited number of cross sectional echocardiographic views will provide accurate left ventricular mass estimates.

Poliner LR, Dehmer GJ, Lewis SE, Parkey RW, Blomqvist CG, and Willerson JT. Left ventricular performance in normal subjects: a comparison of the responses to exercise in the upright and supine positions. ***Circulation*** **1980;62(3):528-534.** Left ventricular (LV) performance at rest and during multilevel exercise in the supine and upright positions was studied in seven normal subjects with equilibrium radionuclide ventriculography. The mean left ventricular end-diastolic volume (LVEDV) during supine rest was 107 ± 10 ml (± SEM) and 85 ± 6 ml ($p < 0.02$) in the upright position; the mean resting left ventricular end-systolic volumes (LVESV) were not different in the upright and supine positions. The LV ejection fraction (LVEF) tended to be slightly higher in the supine (76 ± 2%) than in the upright position (72 ± 4%). The resting heart rate was 89 ± 5 beats/min upright, compared with 71 ± 6 beats/min supine ($p < 0.05$). Multilevel exercise testing was carried out at a low work load of 300 kpm/min, an intermediate work load of 600-750 kpm/min and a peak work load of 1092 ± 66 kpm/min supine and 946 ± 146 kpm/min upright ($p < 0.05$). With peak exercise, supine LVEDV increased significantly, to 135 ± 13 ml (27%), but LVESV did not change. LVEF increased from 76 ± 2% to 84 ± 2% ($p < 0.05$). With upright exercise, LVEDV increased 39% above the resting level, to 116 ± 8 ml ($p < 0.02$), but remained lower than the supine LVEDVs at intermediate ($p < 0.05$) and peak work loads. LVESV decreased significantly by 41%, to 19 ± 3 ml, and was significantly smaller than the corresponding supine volume at intermediate and peak exercise ($p < 0.05$). LVEF increased from 72 ± 4% to 91 ± 2% ($p < 0.05$), which was significantly higher than peak supine LVEF ($p < 0.05$). Heart rates at rest and during exercise were higher in the upright position ($p < 0.05$), but arterial pressures and double products did not differ significantly.

Measurements of LV volumes at rest and during exercise in both the supine and upright positions by dynamic radionuclide scintigraphy suggest that stroke volume during exercise is maintained by a combination of the Frank-Starling mechanism and an enhanced contractile state.

Raven PB, Pape G, Taylor WF, Gaffney FA, and Blomqvist CG. Hemodynamic changes during whole body surface cooling and lower body negative pressure. ***Aviat Space Environ Med*** **1981;52(7):387-391.** Six young healthy male subjects were studied to evaluate the use of whole body surface cooling (WBSC) as an antiorthostatic intervention. Previous studies in our laboratory have demonstrated that perfusion of an Apollo cooling garment with 16°C water produced a significant increase in stroke volume and decrease in

heart rate at rest and during lower body negative pressure (LBNP). However, optimal perfusion temperatures have not been determined. The present study examined the effects of WBSC using perfusion of water at a temperature of 10°C. This perfusion temperature produced a greater decrease in mean skin temperature (T_{sk}) than water at 16°C, -4°C drop compared to -2°C respectively. The hemodynamic effects were also more prominent with 10°C water as shown by the increase in stroke volume of 11% at rest and of 35% during LBNP at -50 torr compared to control measurements at ambient temperature. Heart rates were lowered significantly (8 beats/min) and systolic arterial blood pressure was higher (8 torr). Cooling with 10°C water produced a slight increase in muscle tone, reflected by a small but significant increase (+84 ml/min) in oxygen uptake. These data suggest that WBSC is an effective nonpharmacologic means of controlling preload and deserves further investigation as an antiorthostatic intervention.

Raven PB, Rohm-Young D, Blomqvist CG. Physical fitness and cardiovascular response to lower body negative pressure. *J Appl Physiol* 1984;56(1):138-144. Fourteen young male volunteers (mean age 28.1 yr) underwent maximal exercise performance testing and lower body negative pressure (LBNP) challenge to -50 Torr. Two distinct groups, fit (F, n = 8), mean maximal aerobic capacity (VO_{2max}) = 70.2 ± 2.6 (SE) ml O_2 kg^{-1} •min^{-1}, and average fit (AF, n = 6), mean VO_{2max} = 41.3 ± 2.9 ml O_2 kg^{-1}• min^{-1}, $P < 0.001$, were evaluated. Rebreathing CO_2 cardiac outputs, heart rate (HR), blood pressure (BP), and leg circumference changes were monitored at each stage of progressive increases in LBNP to -50 Torr. The overall hemodynamic responses of both groups of subjects to LBNP were qualitatively similar to previous findings. There were no differences between F and AF in peripheral venous pooling as shown by a leg compliance (Δ leg volume/ Δ LBNP) for the F of 12.6 ± 1.1 and for the AF 11.6 ± 2.0, $P > 0.05$. The F subjects had significantly less tachycardic response [Δ HR/ Δsystolic BP of F = 0.7 beats/Torr] to LBNP to -50 Torr than the AF subjects [Δ HR/Δ systolic BP of unfit (UF) = 1.36 beats/Torr], $P < 0.05$. In addition, overall calculated peripheral vascular resistance was significantly higher in the AF subjects ($P < 0.001$), and there was a more marked decrease in systolic BP of the F subjects between the LBN pressures of -32 to -50 Torr. We concluded that the reflex response to central hypovolemia was altered by endurance exercise training.

Raven PB, Saito M, Gaffney FA, Schutte J, and Blomqvist CG. Interactions between surface cooling and LBNP-induced central hypovolemia. *Aviat Space Environ Med* 1980;51(5):497-503. The interaction between whole body surface cooling (WBSC) and progressive lower body negative pressure (LBNP) to -50 torr was evaluated in nine healthy male volunteers, mean age 29 ± 1.7 years. WBSC, accomplished by circulating 16°C water through an Apollo cooling garment, produced a significant drop in mean skin temperature of 1.96°C ($p<0.001$). Cardiac output (Q) was measured by the C_2H_2 rebreathing technique. Changes in leg volume (LgV) were monitored by a Whitney strain gauge. WBSC at rest produced a significant decrease in leg volume of 0.27 1 ($p<0.01$). Heart rate decreased (-7 bpm, $p<0.01$) and systolic arterial blood pressure was increased (+6 torr, $p<0.02$). The hemodynamic effects of cooling were maintained throughout progressive levels of LBNP with consistently lower leg volumes and heart rates and higher stroke volumes and systolic pressure ($p<0.01$ for all measurements). The data suggest that WBSC produces a central displacement of cutaneous venous volume resulting in an increase in stroke volume.

Roberts LA, Slocum GR, and Riley DA. Morphological study of the innervation pattern of the rabbit sinoatrial node. *Am J Anat* 1989;185:74-88. The pattern of nerves, ganglia, and fine nerve processes in the adult rabbit sinoatrial node, identified by microelectrode recording was defined by staining histochemically for cholinesterase followed by silver impregnation. A generalized repeatable pattern of innervation was recognized, including 1) a large ganglionic complex inferior to the sinoatrial node; 2) two or three moderately large nerves traversing the sinoatrial node parallel to the crista terminalis; 3) nerves entering the region from the atrial septum, the superior vena cava, and the inferior vena cava; and 4) a fine network of

nerve processes, particularly extensive in the morphologically dense small-cell part of the sinoatrial node. When the site of initial depolarization in the node was located and marked by a broken-off electrode tip, it was found, after cholinesterase staining, to be characterized by a cluster of cells enclosed in a nest or basket of fine nerves. Similar nested cell clusters were observed elsewhere in the sinoatrial node in this same preparation and in other hearts. A complex interweaving of atrial muscle fibers was observed medial and inferomedial to the sinoatrial node, which may form the anatomical basis for the lack of conduction through this region. The morphological pattern of nerves, ganglia, and myocardial cells described in this study emphasizes the complexity of innervation of the sinoatrial node, including its intrinsic neural elements. Cholinesterase/silver staining can be useful in the definition and comparison of electrophysiologically identified sites within the sinoatrial node.

Shykoff BE, and Swanson HT. A model-free method for mass spectrometer response correction. *J Appl Physiol* 1987;63(5):2148-2153. A new method for correction of mass spectrometer output signals is described. Response-time distortion is reduced independently of any model of mass spectrometer behavior. The delay of the system is found first from the cross-correlation function of a step change and its response. A two-sided time-domain digital correction filter (deconvolution filter) is generated next from the same step response data using a regression procedure. Other data are corrected using the filter and delay. The mean squared error between a step response and a step is reduced considerably more after the use of a deconvolution filter than after the application of a second-order model correction. O_2 consumption and CO_2 production values calculated from data corrupted by a simulated dynamic process return to near the uncorrupted values after correction. Although a clean step response or the ensemble average of several responses contaminated with noise is needed for the generation of the filter, random noise of magnitude $\leq 0.5\%$ added to the response to be corrected does not impair the correction severely.

Snell PG, Martin WH, Buckey JC, and Blomqvist CG. Maximal vascular leg conductance in trained and untrained men. *J Appl Physiol* 1987;62(2):606-610. Lower leg blood flow and vascular conductance were studied and related to maximal oxygen uptake in 15 sedentary men (28.5 ± 1.2 yr, mean ± SE) and 11 endurance-trained men (30.5 ± 2.0 yr). Blood flows were obtained at rest and during reactive hyperemia produced by ischemic exercise to fatigue. Vascular conductance was computed from blood flow measured by venous occlusion plethysmography, and mean arterial blood pressure was determined by auscultation of the brachial artery. Resting blood flow and mean arterial pressure were similar in both groups (combined mean, 3.0 ml•min^{-1}•100 ml^{-1} and 88.2 mmHg). After ischemic exercise, blood flows were 29- and 19-fold higher ($P<0.001$) than rest in trained (83.3 ± 3.8 ml•min^{-1}•100ml^{-1}) and sedentary subjects (61.5 ± 2.3 ml•min^{-1}•100ml^{-1}), respectively. Blood pressure and heart rate were only slightly elevated in both groups. Maximal vascular conductance was significantly higher ($P<0.001$) in the trained compared with the sedentary subjects. The correlation coefficients for maximal oxygen uptake vs. vascular conductance were 0.81 (trained) and 0.45 (sedentary). These data suggest that physical training increases the capacity for vasodilation in active limbs and also enables the trained individual to utilize a larger fraction of maximal vascular conductance than the sedentary subject.

Sprenkle JM, Eckberg DL, Goble RL, Schelhorn JJ, and Halliday HC. Device for rapid quantification of human carotid baroreceptor-cardiac reflex responses. *J Appl Physiol* 1986;60(2):727-732. We designed, constructed, and evaluated a new device to characterize the human carotid baroreceptor-cardiac reflex response relation rapidly. We designed this system for study of reflex responses of astronauts before, during, and after space travel. The system comprises a new tightly sealing silicone rubber neck chamber, a stepping motor-driven electro-deposited nickel bellows pressure system, capable of delivering sequential

R-wave-triggered neck chamber pressure changes between +40 and -65 mmHg, and a microprocessor-based electronics system for control of pressure steps and analysis and display of responses. This new system provokes classic sigmoid baroreceptor-cardiac reflex responses with threshold, linear, and saturation ranges in most human volunteers during one held expiration.

Tomioka S, Kubo S, Guy HJB, and Prisk GK. Gravitational independence of single-breath washout in recumbent dogs. *J Appl Physiol* 1988;64(2):642-648. To examine the mechanisms of lung filling and emptying, Ar-bolus and N_2 single-breath washout tests were conducted in 10 anesthetized dogs (prone and supine) and in three of those dogs with body rotation. Transpulmonary pressure was measured simultaneously, allowing identification of the lung volume above residual volume at which there was an inflection point in the pressure-volume curve (V_{IP}). Although phase IV for Ar was upward, phase IV for N_2 was small and variable, especially in the prone position. No significant prone to supine differences in closing capacity for Ar were seen, indicating that airway closure was generated at the same lung volumes. The maximum deflections of phase IV for Ar and N_2 from extrapolated phase III slopes were smaller in the prone position, suggesting more uniform tracer gas concentrations across the lungs. V_{IP} was smaller than the closing volume for Ar, which is consistent with the effects of well-developed collateral ventilation in dogs. Body rotation tests in three dogs did not generally cause an inversion of phase III or IV. We conclude that in recumbent dogs regional distribution of ventilation is not primarily determined by the effect of gravity, but by lung, thorax, and mediastinum interactions and/or differences in regional mechanical properties of the lungs.

West JB, Guy HJ, Gaines RA, Hill PM, and Wagner PD. Battery of single-breath tests for rapid measurement of pulmonary function using a respiratory mass spectrometer. *Pneumologie* 1975;151(4):258-263. Modern respiratory mass spectrometers are much improved with respect to their reliability and flexibility. In addition, small dedicated computers are now available for the rapid analysis of data. We describe here a package of single-breath tests utilizing a respiratory mass spectrometer, flow and volume meters, and a small dedicated computer which allows a considerable amount of information about pulmonary function to be obtained in a non-invasive manner in a short time. The principle of the measurement is that the patient performs a series of respiratory maneuvers in approximately 10 min and all data analysis is done by computer. Such a battery of single-breath tests might be valuable in a general hospital setting for the screening of large numbers of patients.

The initial stimulus for investigating a series of single breath tests was provided by the National Aeronautics and Space Administration who were interested in a non-invasive series of lung function tests which could be used to measure the pulmonary function of orbiting astronauts. The package that I am about to describe was originally developed for this purpose and we hope it will fly in the shuttle at the end of this decade. However, the battery has also been set up in one of the University hospitals where it is being used to collect data on patients with minimal amounts of lung disease.

RENAL AND ENDOCRINE

Leach CS. Fluid control mechanisms in weightlessness. *Aviat Space Environ Med* 1987;58(9,Suppl):A74-A79. Experiments performed on Space Shuttle flights have emphasized study of the earliest effects of the cephalad fluid shift resulting from microgravity. Analysis of one subject's urine collected during flight showed that a sharp increase in antidiuretic hormone occurred within 2 h of launch, followed by an increase in cortisol excretion. Although this subject had symptoms of the space adaptation syndrome (SAS), inflight data from Spacelab missions suggested that these transient changes were not caused by SAS. Unpaired *t*-tests and Mann-Whitney tests showed that before and after flight, plasma thyroxine and urine osmolality were significantly higher in Shuttle crewmembers who exhibited more severe symptoms of SAS than in asymptomatic crewmembers. Collection of inflight data from more crewmembers should allow distinction between the effects of SAS and effects of weightlessness, and in the future several additional fluid regulation hormones will be measured in samples from crewmembers for a more complete understanding of fluid control during weightlessness.

Leonard JI. Understanding metabolic alterations in space flight using quantitative models: fluid and energy balance. *Acta Astronautica* 1986;13(6/7):441-457. This report summarizes many of the results obtained during the Skylab program, on metabolic changes during weightlessness. The examination of the data was conducted following an integrated multi-disciplinary and multi-experimental approach. Emphasis is given on several major aspects of metabolic adaptation to space flight: fluid-electrolyte regulation, mechanisms of hormone disturbances, energy balance and etiology of weight loss. The aim is to obtain a composite picture of the fluid, electrolyte and energy response to weightlessness.

BONE AND CALCIUM

Arnaud CD. Role of dietary calcium in osteoporosis. *Adv Intern Med* 1990;35:93-106. Osteoporosis largely affects white women above the age of 65 years. This, coupled with the fact that life expectancy of this population is now approaching an average of nine decades in the United States, implies that the clinical consequences of osteoporosis will afflict a large and increasing percentage of the internist's patients. In fact, approximately 40% of women will have suffered from wrist or vertebral fractures secondary to osteoporosis after they reach age 65. In his extensive review of this important problem, Dr. Claude Arnaud addresses the critical question of the role of calcium intake both in preventing and treating osteoporosis. This is a controversial issue which requires the very balanced discussion that Dr. Arnaud has provided for the epidemiologic and clinical studies that have addressed this question. There are now good data showing a relationship between low calcium intake in various countries and the incidence of osteoporotic fractures. The data on calcium supplementation, albeit primarily in nonrandomized studies, however, show an inconsistent effect on bone density. Finally, the only study examining the effect of added dietary calcium on fracture prevalence concluded that calcium supplementation reduced the number of fractures by one half. While estrogen replacement remains the most effective means of preventing osteoporosis, Dr. Arnaud concludes that while our knowledge in this field is still rudimentary, for many women calcium supplementation represents a reasonable and safe means of attempting to prevent osteoporosis.

Arnaud CD, and Kolb FO. The calciotropic hormones & metabolic bone disease. In: BASIC AND CLINICAL ENDOCRINOLOGY (Ed.) Greenspan FS. East Norwalk: Appleton & Lange, 1991;247-322.

Arnaud CD, Tsao HS, Littledike T, Hess J, Laakso K, and Bischoff J. Radioimmunoassay of human parathyroid hormone in serum. *J Clin Invest* 1971;50:21-34. A new radioimmunoassay for human parathyroid hormone (PTH) in serum, which can measure the hormone present in 94% of the normal sera tested, is described. It is based on the ability of human PTH to compete with ^{131}I-labeled bovine PTH for binding to an antiserum directed against porcine PTH. This antiserum distinguishes between human PTH extracted from parathyroid adenomata and that present in hyperparathyroid sera. Evidence is given to suggest that this is due to immunochemical changes in the hormone extracted from adenomata and not to immunochemical heterogeneity of the hormone present in serum.

Physiologic data supporting the validity and specificity of the assay are presented. Induced episodes of hypercalcemia and hypocalcemia resulted in appropriate responses in serum immunoreactive PTH (IPTH) in normal subjects and in patients with Paget's disease of bone. In normals, there was a progressive increase in serum IPTH in the late afternoon and evening, suggesting a diurnal secretory rhythm. A negative correlation was found between the serum calcium and serum IPTH over the normal range of serum calcium values; a positive correlation was found between these variables in patients with primary hyperparathyroidism. There was apparent overlap between serum IPTH values in normal subjects and patients with primary hyperparathyroidism, but formal discriminate analysis of values for serum calcium and IPTH demonstrated separation of these two groups, without overlap.

Bikle DD, Halloran BP, Cone CM, Globus RK, Morey-Holton E. The effects of simulated weightlessness on bone maturation. *Endocrinology* 1987;120(2):678-684. In earlier studies we showed that elevating the hind limbs of growing rats for up to 2 weeks results in a temporary cessation of bone growth in the hind limbs and a transient fall in the serum levels of 1,25-dihydroxyvitamin D. To determine whether such skeletal unloading also retards the maturation of bone, as seen in vitamin D-deprived animals, we fractionated by density the tibiae from rats whose hind limbs had been elevated for up to 15 days. These fractions were analyzed for dry weight, calcium content, and calcium and proline uptake. The most dense fraction (fraction 4) had the highest degree of mineralization (ratio of calcium to dry weight) and comprised

82% of the total dry weight of the control tibiae. The total incorporation of [^{3}H]proline administered *in vivo* 24 h before removing the tibiae was evenly distributed among all of the fractions, although it was highest in the least dense fraction (fraction 1) when normalized to dry weight. Total incorporation of ^{45}Ca was highest in fraction 4, although when normalized to dry weight it was highest in fraction 3. With skeletal unloading, the portions of bone and ^{45}Ca incorporation in fraction 4 decreased, while the proportions in less dense fractions increased. [^{3}H]Proline incorporation fell in all fractions. These effects were maximal after 10 days of unloading and returned toward the control values after that time. We conclude that skeletal unloading transiently reduced bone formation and retarded mineralization in the growing rat, which resulted in a decrease in mature bone.

Cann CE. Quantitative CT for determination of bone mineral density: a review. *Radiology* 1988;166(2):509-522. One of the major uses of quantitative computed tomography (CT) has been the measurement of bone mineral density (BMD) at various skeletal sites. The published literature on this subject from 1974 to the present is extensive. Because many investigators and clinicians are just now starting to explore the utility of this technique, the author reviewed this literature to provide both the historic perspective and current status of BMD measurement with CT. The physical and physiologic bases of the method, accuracy, reproducibility, radiation dose, and clinical utility are all discussed.

Cann CE, Henzl M, Burry K, Andreyko J, Hanson F, Adamson GD, Trobough G, Henrichs L, and Stewart G. Reversible bone loss is produced by the GnRH agonist Nafarelin. In: CALCIUM REGULATION AND BONE METABOLISM: BASIC AND CLINICAL ASPECTS, Vol. 9, (Eds.) Cohn DV, Martin TJ, and Meunier PJ. New York: Elsevier Science Publishers B.V., 1987;9:123-127.

Cavanaugh DJ, and Cann CE. Brisk walking does not stop bone loss in postmenopausal women. *Bone* 1988;9:201-204. The rate of loss of spinal trabecular mineral density (TMD) in postmenopausal women, 49-64 years, was measured during a 52 week walking program. The 8 women who walked were 5.6 ± 4.4 years past menopause (mean ± SD) compared to 6.5 ± 5.1 for 9 nonwalkers. Walkers participated in a progressive walking program for 15-40 min at a heart rate of between 60-85% of maximal age adjusted heart rate, 3 days per week for 52 weeks. Spinal trabecular mineral density was measured using quantitative computed tomography at entry, 6 and 12 months. Pre-exercise heart rate in the walkers decreased 7.8 ± 1.7 beats per min (mean ± SEM)(p<0.01) from week 0 to week 52, while post-exercise heart rate did not change. Initial spinal mineral density in the walkers was 114 ± 18 mg/cm^3 (mean ± SD) and 98 ± 19 mg/cm^3 in the controls (NS). Bone loss was 5.6 ± 1.4% (mean ± SEM) in the walkers and 4.0 ± 1.2% in the controls; both of these losses were significantly different from zero (p<0.005, p<0.01, respectively), but they were not different from each other. Our study shows that a moderate brisk walking program of one year duration does not prevent the loss of spinal bone density in early-postmenopausal women.

Doty SB. Cell-to-cell communication in bone tissue. In: THE BIOLOGICAL MECHANISMS OF TOOTH ERUPTION AND ROOT RESTORATION (Ed.) Davidovitch Z. Birmingham: EBSCO Media, 1988;61-69. Communication between bone cells is necessary in order to synthesize bone matrix in an organized and structurally responsive manner. Networks of cell-cell communication are morphologically described which include gap junctions and cytoskeletal networks between bone cells, as well as nerve fiber endings in the periosteum which make contact with the periosteal cells. These periosteal cells are coupled, in turn, with adjacent bone cells throught gap junctional complexes.

Doty SB, Morey-Holton E, Durnova GN, and Kaplansky AS. Cosmos 1887: morphology, histochemistry, and vasculature of the growing rat tibia. ***FASEB J*** **1990;4:16-23.** Light microscopy, electron microscopy, and enzyme histochemistry were used to study the effects of spaceflight on metaphyseal and cortical bone of the rat tibia. Cortical cross-sectional area and perimeter were not altered by a 12.5-day spaceflight in 3-month-old male rats. The endosteal osteoblast population and the vasculature near the periosteal surface in flight rats compared with ground controls showed more pronounced changes in cortical bone than in metaphyseal bone. The osteoblasts demonstrated greater numbers of transitional Golgi vesicles, possibly caused by a decreased cellular metabolic energy source, but no difference in the large Golgi saccules or the cell membrane-associated alkaline phosphatase activity. The periosteal vasculature in the diaphysis of flight rats often showed lipid accumulations within the lumens of the vessels, occasional degeneration of the vascular wall, and degeneration of osteocytes adjacent to vessels containing intraluminal deposits. These changes were not found in the metaphyseal region of flight animals. The focal vascular changes may be due to ischemia of bone or a developing fragility of the vessel walls as a result of spaceflight.

Fielder PJ, Morey ER, and Roberts WE. Osteoblast histogenesis in periodontal ligament and tibial metaphysis during simulated weightlessness. ***Aviat Space Environ Med*** **1986;57(12):1125-1130.** According to nuclear size, fibroblast-like cells adjacent to bone surfaces in the periodontal ligament (PDL) and tibial primary spongiosa (PS) were classified as less differentiated progenitors and committed osteoprogenitors (A/A'), nonosteogenic cells (B), or preosteoblasts (C/D). The ratio of A/A' to C/D cells reflects osteogenic status of bone lining tissue. When 83-day-old rats were subjected to simulated weightlessness (S-W) for 17 d and examined for changes in osteoblast histogenesis, PDL and PS cell populations increased in A/A' cells ($p<0.01;<0.05$) but decreased in C/D cells ($p<0.01;<0.05$) compared to controls. These data indicate that the nuclear volume method, originally developed in PDL, can also be used to assess osteoblast histogenesis in PS of long bones, and that simulated weightlessness in the present experimental context interferes with osteoblast histogenesis. Since the surfaces of both weightbearing (PS) and nonweightbearing (PDL) bones were affected, systemic factors appear important in the gravity-related mechanism of osteoblast histogenesis. Although unloading of the tibia and cephalad fluid shifts occur during S-W, the data attained in this experiment could also be explained by stress and/or cessation of growth in the S-W rats.

Garetto LP, Gonsalves MR, Morey ER, Durnova G, and Roberts WE. Preosteoblast production 55 hours after a 12.5-day spaceflight on Cosmos 1887. ***FASEB J*** **1987;4:24-28.** The influence of 12.5 days of spaceflight and a 55 h stressful recovery period (at 1 *g*) on fibroblastlike osteoblast precursor cells was assessed in the periodontal ligament (PDL) of rats that were 91 days old at launch. Nuclear morphometry was used as a marker for precursor cell differentiation in 3 μm sections cut in the midsagittal plane from the maxillary first molar. According to nuclear volume, cells were classified as preosteoblasts (C + D cells, $\geq 120 \mu m^3$) and less differentiated progenitor cells (A + A' cells, 40-79 μm^3). Compared with synchronous controls (simulated flight conditions), the 55 h postflight recovery period at 1 *g* resulted in a 40% decrease in the A + A' cell population, a 42% increase in the C + D cells, and a 39% increase in the number of PDL fibroblastlike cells near the bone surface. These results are consistent with a postflight osteogenic response in PDL. This recovery response occurred despite physiological stress in the flight animals that resulted in a highly significant ($P \leq 0.001$) increase in adrenal weight. The data suggest that after spaceflight there is a strong and rapid recovery mechanism for osteoblast differentiation that is not suppressed by physiological stress.

Globus RK, Bikle DD, Halloran B, and Morey-Holton ER. Skeletal response to dietary calcium in a rat model simulating weightlessness. *J Bone Miner Res* 1986;1(2):191-197. Unweighting the hindlimbs of a rat by tail suspension leads to a decrease in bone in the unweighted hindlimbs, but not in the normally weighted forelimbs. We evaluated whether increments in dietary calcium could prevent this. Growing rats were fed diets ranging in calcium content from 0.1% to 2.4%. After the rats were suspended for two weeks, we found no differences between suspended and control animals fed the same diet with respect to calcium transport or serum levels of calcium, phosphorus, 1,25-dihydroxyvitamin D, and parathyroid hormone. In both groups, increasing dietary calcium reduced active intestinal calcium transport and serum 1,25-dihydroxyvitamin D levels. The calcium content of the tibia and lumbar vertebra (but not the humerus) was reduced in suspended rats compared to control rats fed the same diet. However, increasing dietary calcium increased the calcium content of all bones in both suspended and control animals. The bone formation rate at the tibiofibular junction (measured by double-label tetracycline) was reduced in the suspended animals compared to controls and was not altered by dietary calcium. However, the marrow area of the tibia, an indication of bone resorption, did not differ between suspended and control animals and was equally reduced in both groups when dietary calcium was increased. Our data suggest that the deleterious effects of skeletal unweighting on bone formation cannot be explained by changes in the calciotropic hormones and are not reversed by increments in dietary calcium. However, increasing dietary calcium can increase bone calcium, even in unweighted limbs, by decreasing bone resorption.

Globus RK, Bikle DD, and Morey-Holton E. The temporal response of bone to unloading. *Endocrinology* 1986;118(2):733-742. A model of weightlessness in which the hindlimbs of rats are elevated by their tails at a 40° angle to unload the hindlimbs while maintaining normal weight bearing on the forelimbs has been used to simulate certain conditions of spaceflight. When we used this model in growing rats, we found that growth in bone weight ceased by 1 week in the hindlimbs and lumbar vertebrae, whereas growth in bone weight in the forelimbs and cervical vertebrae remained unaffected. Within 2 weeks, however, the accretion of bone weight in the hindlimbs and lumbar vertebrae returned to normal despite continued skeletal unloading.

Since bone weight in the growing rat is primarily determined by bone formation (bone resorption is modest), we investigated the effects of selective skeletal unloading on bone formation during 2 weeks of hindlimb elevation using radioisotope incorporation (with ^{45}Ca and [^{3}H]proline) and histomorphometry (with tetracycline labeling). The studies using radioisotope incorporation showed that bone formation was inhibited by the fifth day of skeletal unloading. By the 10th to 12th day, bone formation had returned toward normal. In comparison with cortical bone, cancellous bone (lumbar vertebrae and proximal tibiae) incorporated more ^{45}Ca and [^{3}H]proline (indicating greater metabolic activity) and had a greater absolute response to skeletal unloading. The results of these studies were confirmed by histomorphometric measurements of bone formation using triple tetracycline labeling.

We conclude that this model of simulated weightlessness results in an initial inhibition of bone formation in the unloaded bones. This temporary cessation of bone formation is followed by a cessation in the accretion of bone weight, which then resumes at a normal rate by 14 days despite continued skeletal unloading. We believe that this cycle of inhibition and resumption of bone formation has profound implications for understanding bone dynamics during space flight, immobilization, or bed rest and offers an opportunity to study the hormonal and mechanical factors that regulate bone formation.

Halloran BP, Bikle DD, Wronski TJ, Globus RK, Levens MJ, and Morey-Holton E. The role of 1,25-dihydroxyvitamin D in the inhibition of bone formation induced by skeletal unloading. *Endocrinology* 1986;118(3):948-954. Skeletal unloading results in osteopenia. To examine the involvement of vitamin D in this process, the rear limbs of growing rats were unloaded, and alterations in bone calcium and bone

histology were related to changes in serum calcium (Ca), inorganic phosphorus, 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D [24,25-$(OH)_2D$], and 1,25-dihydroxyvitamin D [1,25-$(OH)_2D$]. Acute skeletal unloading induced a transitory inhibition of Ca accumulation in unloaded bones. This was accompanied by a transitory rise in serum Ca, a 21% decrease in longitudinal bone growth ($P<0.01$), a 32% decrease in bone surface lined with osteoblasts ($P<0.05$), no change in bone surface lined with osteoclasts, and a decrease in circulating 1,25-$(OH)_2D$ from 130 ± 10 to 53 ± 11 pg/ml. No significant changes in the serum concentrations of inorganic phosphorus, 25-hydroxyvitamin D, or 24,25-$(OH)_2D$ were observed. After 2 weeks of unloading, bone Ca stabilized at approximately 70% of control values, and serum Ca and 1,25-$(OH)_2D$ returned to control values. Maintenance of a constant serum 1,25-$(OH)_2D$ concentration by chronic infusion of 1,25-$(OH)_2D$ (Alza osmotic minipump) throughout the study period did not prevent the bone changes induced by acute unloading. These results suggest that acute skeletal unloading in the growing rat produces a transitory inhibition of bone formation, which, in turn, produces a transitory hypercalcemia, leading to a temporary decrease in serum 1,25-$(OH)_2D$. No evidence could be found for a direct involvement of 1,25-$(OH)_2D$ in the bone changes induced by skeletal unloading.

Jee WSS, Wronski TJ, Morey ER, and Kimmel DB. Effects of spaceflight on trabecular bone in rats. ***Am J Physiol*** **1983;244:R310-R314.** Alterations in trabecular bone were observed in growing male Wistar rats after 18.5 days of orbital flight on the COSMOS 1129 biosatellite. Spaceflight induced a decreased mass of mineralized tissue and an increased fat content of the bone marrow in the proximal tibial and humeral metaphyses. The osteoblast population appeared to decline immediately adjacent to the growth cartilage-metaphyseal junction, but osteoclast numbers were unchanged. These results suggested that bone formation may have been inhibited during spaceflight, but resorption remained constant. With the exception of trabecular bone mass in the proximal tibia, the observed skeletal changes returned to normal during a 29-day postflight period.

Morey-Holton ER, and Cone CM. Bone as a model system to organ/tissue responses to microgravity. In: FUNDAMENTALS OF SPACE BIOLOGY (Eds.) Asashima M, and Malacinski GM. Tokyo: Japan Science Society Press, 1990;113-122.

Morey-Holton ER, Schnoes HK, DeLuca HF, Phelps ME, Klein RF, Nissenson RH, and Arnaud CD. Vitamin D metabolites and bioactive parathyroid hormone levels during Spacelab 2. ***Aviat Space Environ Med*** **1988;59(11):1038-1041.** The purpose of this study was to determine whether plasma levels of the vitamin D hormone and parathyroid hormone (PTH), two potent activators of bone remodeling sites, were altered in four astronauts during the 8-day (d) Spacelab 2 mission (SL2). Increased circulating levels of either hormone could change calcium homeostasis and bone cell activity and, thus contribute to bone loss in crewmembers in space. The vitamin D hormone was elevated in all astronauts at the end of the first inflight day but returned to normal by the seventh day. Biologically active PTH tended to be normal throughout the mission. Both hormones were within the normal range by the end of the 8-d flight of this SL2 crew. Plasma levels of 25OHD, 24,25$(OH)_2D$, calcium, phosphorus, and albumin were essentially normal during the mission.

Nissenson RA, Karpf D, Bambino T, Winer J, Canga M, Nyiredy K, and Arnaud CD. Covalent labeling of a high-affinity, guanyl nucleotide sensitive parathyroid hormone receptor in canine renal cortex. *Biochem* 1987;26(7):1874-1878. Putative parathyroid hormone (PTH) receptors in canine renal membranes were affinity labeled with ^{125}I-bPTH(1-34) using the heterobifunctional cross-linking reagent *N*-hydroxysuccinimidyl 4-azidobenzoate. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed the presence of a major 85 000 molecular weight (M_r) PTH binding component, the labeling of which was inhibited by nanomolar concentrations of unlabeled PTH and by micromolar concentrations of 5'-guanylyl imidodiphosphate [Gpp-(NH)p]. Labeling was not influenced by the unrelated peptides insulin and arginine vasopressin. Minor PTH binding components of M_r 55 000 and 130 000 were also seen, and labeling of these was likewise sensitive to unlabeled PTH and to Gpp(GH)p. Omission of protease inhibitors during the isolation of plasma membranes resulted in the loss of the M_r 85 000 PTH binding species and the appearance of an M_r 70 000 form. Several minor PTH binding components also were observed. Equilibrium binding studies showed that such membranes had an affinity for PTH indistinguishable from that in membranes isolated with protease inhibitors and displaying a major M_r 85 000 PTH binding species. We conclude that the major form of the adenylate cyclase coupled PTH receptor in canine renal membranes is an M_r 85 000 protein. An endogenous enzyme, probably a lysosomal cathepsin, can cleave this form to produce an M_r 70 000 receptor that retains full functional activity with respect to high-affinity, guanyl nucleotide sensitive PTH binding. The ability to covalently label the PTH receptor in high yield represents a major step toward the structural characterization of this important detector molecule.

Patterson-Buckendahl P, Arnaud SB, Mechanic GL, Martin RB, Grindeland RE, and Cann CE. Fragility and composition of growing rat bone after one week in spaceflight. *Am J Physiol* 1987;252:R240-R246. To gain some insight into the early effects of spaceflight on skeletal metabolism, we quantified the major chemical constituents and a noncollagenous protein, osteocalcin, in the third-lumbar vertebrae and humeri from 8-wk-old rats that were part of the 7-day NASA Spacelab 3 flight experiments. The ratio of calcium to hydroxyproline in the humeral diaphysis increased from 8.5 in preflight to 9.8 in ground simulation control and only to 8.9 in flight bones. There was no demonstrable change in the fraction of nonmineralized collagen. Osteocalcin content was reduced in the humerus and vertebra. Reduced accumulation of mineral and osteocalcin with no associated decrease in collagen in flight animals suggests that both mineralization and collagen metabolism are impaired in growing animals during spaceflight within a few days after launch. Strength tests of the humeri of flight rats showed substantial deficits that appeared to be related, not only to the reduced bone mass, but also to the composition and quality of new bone formed.

Patterson-Buckendahl P, Globus RK, Bikle DD, Cann CE, and Morey-Holton E. Effects of simulated weightlessness on rat osteocalcin and bone calcium. *Am J Physiol* 1989;257:R1103-R1109. Some of the musculoskeletal changes that occur in growing rats during spaceflight are simulated by a model that selectively unloads the hindlimbs while maintaining normal weight bearing on the forelimbs. Using this model we studied the response of mineral and the mineral-binding protein osteocalcin (OC) in the third lumbar vertebra (L_3) and the femoral midshaft to periods of unweighting from 2 to 28 days. Serum OC decreased by 25%, consistent with a decreased rate of bone growth, during the first week of suspension and returned toward control values after 15 days. The L_3 and femur weighed 20% less than control bones after 10-28 days. OC content of L_3 and femur diaphysis were lower after 7 days of suspension and returned to normal levels at 28 days, whereas Ca content rose slightly at 5 days then decreased sharply. OC:Ca ratio was also affected. The data suggest that unweighting affects formation and deposition of OC and Ca differently depending on bone location and duration of unweighting. Both serum and bone OC are highly sensitive indicators of disruption of osteoblast activity by altered skeletal loading.

Roberts WE, and Morey ER. Proliferation and differentiation sequence of osteoblast histogenesis under physiological conditions in rat periodontal ligament. *Am J Anat* 1985;174:105-118. To define the mechanism of osteoblast histogenesis, nuclear morphometry was utilized as a marker for precursor cell differentiation. One hour after ^{3}H-thymidine injection, groups of 7-week old rats were killed at hourly intervals over one complete 24-hr photoperiod (LD 12:12). S-phase and mitosis were assessed in autoradiographs of 3-μm sections of molar periodontal ligament (PDL) adjacent to a physiological bone-forming surface. Labeled nuclei were divided into four categories according to morphometry of nuclear sizes: **A** (40-79 μm^3), **B** (80-119 μm^3), **C** (120-169 μm^3), and **D** ($\geq$ 170 μm^3) cells. **C** and **D** cells synthesize DNA during the light and divide in the following dark phase; the rhythm for **A** cells is the opposite. **B** cells demonstrated no preference and were subsequently determined to be nonosteogenic. Compared to **A** cells the S-phase photoperiod of **C** and **D** cells (combined) is approximately a one-to-one reciprocal relationship, suggesting two proliferating progenitors in series. Based on arrest points in the histogenesis sequence, five compartments are defined: 1) **A'** cells, less differentiated, self-perpetuating precursors; 2) **A** cells, committed osteoprogenitors; 3) **C** cells, G_1 stage preosteoblasts; 4) **D** cells, G_2 stage preosteoblasts; 5) **Ob** cells, morphologically distinct osteoblasts. Minimal elapsed time for the **A** $\rightarrow$ **A'** $\rightarrow$ **C** $\rightarrow$ **D** $\rightarrow$ **Ob** sequence is about 60 hr (five alternating dark/light cycles). A stress/strain-mediated increase in nuclear volume (**A'** $\rightarrow$ **C**) is an important, rate-limiting step in osteoblast differentiation.

Roberts WE, Fielder PJ, Rosenoer LML, Maese AC, Gonsalves MR, and Morey ER. Nuclear morphometric analysis of osteoblast precursor cells in periodontal ligament, SL-3 rats. *Am J Physiol* 1987;252:R247-R251. Five small (55 days old, 196 ± 5 g)(mean ± SE) and five large (83 days old, 382 ± 4 g) Sprague-Dawley strain, specific pathogen-free rats were exposed to a 7-day spaceflight and 12-h postflight recovery period. As measured in 3-μm sections, periodontal ligament (PDL) fibroblastlike cells were classified according to nuclear size: A + A' (40-79), B (80-119), C (120-169), and D ($\geq$170 μm^3). Since the histogenesis sequence is A $\rightarrow$ A' $\Rightarrow$ C $\rightarrow$ D $\rightarrow$ osteoblast, the relative incidence of A + A' to C + D is an osteogenic index. No difference in A + A' or C + D cells in small rats may reflect partial recovery of preosteoblast formation (A $\Rightarrow$ C) during the 12-h postflight period. Large flight rats demonstrated increased numbers of A+A', indicating an inhibition of preosteoblast formation (A $\Rightarrow$ C). At least in the older group, a 7-day flight is adequate to reduce PDL osteogenic potential (inhibition in PDL osteoblast differentiation and/or specific attrition of C + D cells) that does not recover by 12-h postflight.

Sessions NDV, Halloran BP, Bikle DD, Wronski TJ, Cone CM, and Morey-Holton E. Bone response to normal weight bearing after a period of skeletal unloading. *Am J Physiol* 1989;257:E606-E610. Skeletal unloading in the growing rat induces a temporary inhibition of bone formation and thereby a deficit in bone calcium compared with age-matched, normally loaded animals. To determine whether this deficit can be restored by skeletal reloading we measured bone formation rate at the tibiofibular junction and total bone calcium in the tibia and lumbar vertebra in rats whose hindlimbs were unloaded for 2 wk and then reloaded by return to normal weight bearing. Continuously loaded or unloaded animals were also studied. Skeletal unloading reduced bone formation by 34% and tibial and vertebral calcium by 12 and 22%, respectively. Reloading significantly increased the rates of bone formation and calcium accretion 30-34% above normally loaded animals, and by 2 wk had decreased the deficit in tibial and vertebral calcium by 36 and 23%, respectively. These data indicate that the deficit in bone calcium induced by skeletal unloading in the growing rat can be restored in part by return to normal weight bearing. However, the time required to restore bone calcium exceeds the time required to produce the original calcium deficit.

Shaw SR, Vailas AC, Grindeland RE, and Zernicke RF. Effects of a 1-wk spaceflight on morphological and mechanical properties of growing bone. ***Am J Physiol*** **1988;254:R78-R83.** The morphological and mechanical responses of tibia and humerus were assessed in growing male rats after a 1-wk spaceflight aboard NASA Spacelab 3. In contrast to flights of longer duration, changes in middiaphysial cross-sectional morphology were minimal. Inhibition of longitudinal growth was not found in the tibia but was apparent in the humerus. The normal age-related increase in tibial middiaphysial density was not observed in the flight animals. Three-point bending tests indicated that a 1-wk spaceflight impeded the maturation of bone strength and stiffness, with the effects more pronounced in the tibia than in the humerus. Material property alterations in bone thus overshadowed morphological factors in determining the bone's mechanical response. It is likely that deprivation of normal weight-bearing loads was a major factor contributing to the observed changes, but endocrine and other local factors must also be considered.

Shaw SR, Zernicke RF, Vailas AC, DeLuna D, Thomason DB, and Baldwin KM. Mechanical, morphological and biochemical adaptations of bone and muscle to hindlimb suspension and exercise. ***J Biomechan*** **1987;20(3):225-234.** The influences of weightbearing forces on the structural remodeling, matrix biochemistry, and mechanical characteristics of the rat tibia and femur and surrounding musculature were examined by means of a hindlimb suspension protocol and highly intensive treadmill running. Female, young adult, Sprague-Dawley rats were designated as either normal control, sedentary suspended, or exercise suspended rats. For 4 weeks, sedentary suspended rats were deprived of hindlimb-to-ground contact forces, while the exercise suspended rats experienced hindlimb ground reaction forces only during daily intensive treadmill training sessions. The suspension produced generalized atrophy of hindlimb skeletal muscles, with greater atrophy occurring in predominantly slow-twitch extensors and adductors, as compared with the mixed fiber-type extensors and flexors. Region-specific cortical thinning and endosteal resorption in tibial and femoral diaphyses occurred in conjunction with decrements in bone mechanical properties. Tibial and femoral regional remodeling was related to both the absence of cyclic bending strains due to normal weightbearing forces and the decrease in forces applied to bone by antigravity muscles. To a moderate extent, the superimposed strenuous running counteracted muscular atrophy during the suspension, particularly in the predominantly slow-twitch extensor and adductor muscles. The exercise did not, however, mitigate changes in bone mechanical properties and cross-sectional morphologies, and in some cases exacerbated the changes. Suspension with or without exercise did not alter the normal concentrations of collagen, phosphorus, and calcium in either tibial or femur.

Turner RT, Bell NH, Duvall P, Bobyn JD, Spector M, Morey-Holton E, and Baylink DJ. Spaceflight results in formation of defective bone. ***Proc Soc Exp Biol Med*** **1985;180:544-549.** Growing rats were flown on 19 day spaceflights aboard Cosmos 782 and 936 biosatellites. Spaceflight resulted in a prominent skeletal defect of the periosteal surface of the tibia diaphysis. The defect, termed an arrest line, was approximately 3 μm across and separated the bone formed in space from that formed following spaceflight. The bone matrix at the arrest line region was abnormal in that collagen fibers were preferentially orientated parallel to the periosteal surface. In addition, the bone matrix was hypomineralized. The altered bone was inferior to normal bone in resistance to abrasion and may be partially responsible for the decrease in torsional strength observed after spaceflight.

Vailas AC, Deluna DM, Lewis LL, Curwin SL, Roy RR, and Alford EK. Adaptation of bone and tendon to prolonged hindlimb suspension in rats. ***J Appl Physiol*** **1988;65(1):373-376.** The rat hindlimb suspension model was used to ascertain the importance of ground reaction forces in maintaining bone and tendon homeostasis. Young female Sprague-Dawley rats were randomly assigned to either a suspended or a nonsuspended group. After 28 days, femur bones and patellar tendons were obtained for morphological and biochemical analyses. Prolonged suspension induced a significant change in the geometric configuration of the femur middiaphysis by increasing the minimum diameter (12%) without any significant alterations in cortical area, density, mineral, and collagen concentrations. Femur wet weight, length, DNA, and uronic acid concentrations of suspended animals were not significantly different from bones of nonsuspended rats. However, the collagen and proteoglycan concentrations in patellar tendons of suspended rats were 28% lower than the concentrations of matrix proteins in tissues obtained from nonsuspended animals. These data suggest that elimination of ground reaction forces induces alterations in tendon composition and femur diaphyseal shape by changing regional rates in bone remodeling and localized tendon strain. Therefore it appears that ground reaction forces are an important factor in the maintenance of cortical bone and patellar tendon homeostasis during weight-bearing conditions.

Vailas AC, Zernicke RF, Grindeland RE, Kaplansky A, Durnova GN, Li KC, and Martinez DA. Effects of spaceflight on rat humerus geometry, biomechanics, and biochemistry. ***FASEB J*** **1990;4:47-54.** The effects of a 12.5 day spaceflight (Cosmos 1887 biosatellite) on the geometric, biomechanical, and biochemical characteristics of humeri of male specific pathogen-free rats were examined. Humeri of age-matched basal control, synchronous control, and vivarium control rats were contrasted with the flight bones to examine the influence of growth and space environment on bone development. Lack of humerus longitudinal growth occurred during the 12.5 days in spaceflight. In addition, the normal mid-diaphysial periosteal appositional growth was affected; compared with their controls, the spaceflight humeri had less cortical cross-sectional area, smaller periosteal circumferences, smaller anterior-posterior periosteal diameters, and smaller second moments of area with respect to the bending and nonbending axes. The flexural rigidity of the flight humeri was comparable to that of the younger basal control rats and significantly less than that of the synchronous and vivarium controls; the elastic moduli of all four groups, nonetheless, were not significantly different. Generally, the matrix biochemistry of the mid-diaphysial cross sections showed no differences among groups. Thus, the spaceflight differences in humeral mechanical strength and flexural rigidity were probably a result of the differences in humeral geometry rather than material properties.

Vailas AC, Zernicke RF, Matsuda J, Curwin S, and Durivage J. Adaptation of rat knee meniscus to prolonged exercise. ***J Appl Physiol*** **1986;60(3):1031-1034.** The morphological and biochemical adaptations of knee meniscus to prolonged exercise were studied. Female Sprague-Dawley rats maintained under controlled environmental conditions were randomly assigned to either an endurance-trained or a sedentary group. Training consisted of a progressive protocol on a motor-driven treadmill, 5 days/wk for 12 wk. Knee lateral menisci were obtained from anesthetized rats and used for morphological and biochemical analyses. Gastrocnemius succinate dehydrogenase increased 65% in the endurance-trained group, as evidence for a training effect. In the trained group, collagen, proteoglycan, and calcium concentrations increased significantly in the posterior region of the lateral meniscus. In contrast, no significant changes were found in the anterior region of the lateral meniscus. The region-specific changes in meniscal concentrations of calcium and matrix macromolecules in response to prolonged exercise are consistent with the distinctly different mechanical properties and functional roles of the anterior and posterior regions of the rat knee meniscus.

Wronski TJ, and Morey ER. Effect of spaceflight on periosteal bone formation in rats. *Am J Physiol* 1983;244:R305-R309. Male Wistar rats were placed in orbit for 18.5 days aboard the Soviet COSMOS 1129 biological satellite. Tetracycline was administered before and after spaceflight to label areas of bone formation. An inhibition of periosteal bone formation occurred during spaceflight in the tibial and humeral diaphyses, but this defect was corrected during the postflight period. The increased extent of arrest lines at these skeletal sites suggested that periosteal bone formation may have even ceased during spaceflight. The rib exhibited a small but nonsignificant decrease in periosteal bone formation. Endosteal bone resorption was not affected markedly by spaceflight conditions. The observed inhibition of periosteal bone formation may be a result of mechanical unloading, but endocrine factors cannot be ruled out.

Wronski TJ, and Morey-Holton ER. Skeletal response to simulated weightlessness: a comparison of suspension techniques. *Aviat Space Environ Med* 1987;58(1):63-68. The skeletal response to simulated weightlessness was studied in rats subjected to two different methods of suspension. Skeletal unloading of the hind limbs for a two week period was achieved by use of either a back harness or tail traction. In comparison to pair-fed control rats, back-suspended rats failed to gain weight whereas tail-suspended rats exhibited normal weight gain. Quantitative bone histomorphometry revealed marked skeletal abnormalities in the proximal tibial metaphysis of back-suspended rats. Loss of trabecular bone mass in these animals was due to a combination of depressed longitudinal bone growth, decreased bone formation, and increased bone resorption. In contrast, the proximal tibia of tail-suspended rats was relatively normal by these histologic criteria. However, a significant reduction in trabecular bone volume occurred during 2 weeks of tail suspension, possibly due to a transient inhibition of bone formation during the early stages of skeletal unloading. Lack of weight gain in back-suspended rats may be indicative of a pronounced stress response during which corticosteroids adversely affected the skeleton. Maintenance of normal weight gain by tail-suspended rats provides evidence for the less traumatic nature of this method of suspension. Our findings indicate that tail suspension may be a more appropriate model for evaluating the effects of simulated weightlessness on skeletal homeostasis.

Wronski TJ, Morey-Holton ER, Doty SB, Maese AC, and Walsh CC. Histomorphometric analysis of rat skeleton following spaceflight. *Am J Physiol* 1987;252:R252-R255. Male Sprague-Dawley rats were placed in orbit for 7 days aboard the space shuttle. Bone histomorphometry was performed in the long bones and lumbar vertebrae of flight rats and compared with data derived from ground-based control rats. Trabecular bone mass was not altered during the 1st wk of weightlessness. Strong trends were observed in flight rats for decreased periosteal bone formation in the tibial diaphysis, reduced osteoblast size in the proximal tibia, and decreased osteoblast surface and number in the lumbar vertebra. For the most part, histological indexes of bone resorption were normal in flight rats. The results indicate that 7 days of weightlessness are not of sufficient duration to induce histologically detectable loss of trabecular bone in rats. However, cortical and trabecular bone formation appear to be diminished during the 1st wk of spaceflight.

MUSCLE

Curwin SL, Vailas AC, and Wood J. Immature tendon adaptation to strenuous exercise. *J Appl Physiol* 1988;65(5):2297-2301. White Leghorn roosters (3 wk old) were randomly assigned to runner or control groups. Runners were subjected to a progressive treadmill running program for 8 wk, 5 days/wk at 70-80% maximal O_2 consumption (VO_{2max}). After 8 wk, runners showed a significant elevation in gastrocnemius fumarase activity (51%) and a 21 % increase in $VO_{2\,max}$ compared with controls. The exercise program induced a significant increase in tendon collagen deposition (46%) without any changes in DNA, proteoglycan, and collagen concentrations or tendon dry weight. Also, tendon collagen from runners contained fewer (50%) pyridinoline cross-links. These results suggest that high-intensity exercise causes greater matrix-collagen turnover in growing chickens, resulting in reduced maturation of tendon collagen.

Ellis S, Giometti CS, and Riley DA. Changes in muscle protein composition induced by disuse atrophy: analysis by two-dimensional electrophoresis. *Physiologist* 1985;28(6,Suppl):S159-S160. Two dimensional (2-D) electrophoresis has been used extensively to map the major proteins of rabbit, rat, chicken and human muscle. However, it has been little used for investigating possible changes in the broad spectrum of proteins in muscle which has undergone atrophy due to load removal. By this technique it was shown that the myosin light chains and tropomyosins shift from the typical slow-twitch muscle pattern to a fast-twitch pattern after a long duration inactivity of the hind limb muscles of the cat produced by cord transection. Six week immobilization of rat hindlimbs showed no changes by 2-D electrophoresis in the characteristic myosin light chains in either the soleus or vastus lateralis. One and four weeks of immobilization produced significant alterations in myosin light chains and related peptides in 2-D electrophoresis. However, in addition to the reported changes in contractile proteins, there are indications that there may occur substantial changes in the relative quantities, and perhaps even quality, of non-contractile skeletal muscle proteins following disuse atrophy. This was the rationale for undertaking a 2-D electrophoretic analysis of the broad spectrum of muscle proteins in the soleus and EDL muscles from hindlimbs maintained load-free for 10 days.

Fahlman CS, and Riley DA. Colchicine-induced sprouting of the neuromuscular junction in the pigeon extensor digitorum longus muscle. *Brain Res* 1986;363:156-160. Colchicine-induced motor endplate sprouting in the extensor digitorum longus muscle of the pigeon was examined. Ten days after the drug application sprouting from the endplate arborizations and nodes of Ranvier were observed. No concomitant changes in endplate surface area or in the degree of terminal branching could be demonstrated. Similarities between the sprouting patterns of the pigeon endplate and the mammalian endplate are discussed.

Fitts RH, Metzger JM, Riley DA, and Unsworth BR. Models of disuse: a comparison of hindlimb suspension and immobilization. *J Appl Physiol* 1986;60(6):1946-1953. The effects of 1 and 2 wk of hindlimb suspension (HS) on rat skeletal muscle function were determined and the results compared with those obtained previously with hindlimb immobilization (HI). Both models of disuse (HS and HI) primarily affected slow-twitch muscle. Each decreased the isometric twitch duration in the slow-twitch soleus; however, the HS-mediated effect was entirely a result of a shortened contraction time (CT), whereas HI reduced one-half relaxation time ($^1/_2$ RT) as well as CT. Soleus muscle mass and peak tetanic tension (P_0) declined with disuse. The HS effect on muscle mass and P_0 was variable, however, for all experiments HS produced atrophy equal to or greater than HI. A major difference existed in the effects of HS and HI on the maximal speed of soleus muscle shortening (V_{max}). One and 2 wk of HS produced increases in V_{max} to 4.45 $\pm$ 0.34 and 6.83 $\pm$ 0.74 fiber lengths/s, respectively, compared with control velocities of 3.05 $\pm$ 0.08. By contrast over a similar time period, HI had no significant effect on soleus V_{max}. The increase in V_{max} at 14

days of HS was associated with, and perhaps caused by, the increased expression of a second faster migrating isozyme of myosin. The new native isozyme comigrated with fast myosin, but its light chain subunits contained only LC_{1S} and LC_{2S}. The mechanism responsible for the increase is unknown. One plausible explanation is that the apparent HS-mediated modification in muscle fiber type is dependent on the elimination of loadbearing or isometric contractions, a condition that does not exist during HI.

Hoh JFY, Hughes S, Hale PT, and Fitzsimons RB. Immunocytochemical and electrophoretic analyses of changes in myosin gene expression in cat limb fast and slow muscles during postnatal development. ***J Muscle Res Cell Motil*** **1988;9:30-47.** Changes in myosin synthesis during the postnatal development of the fast extensor digitorum longus (EDL) and the slow soleus muscles of the kitten were examined using immunocytochemical techniques supplemented by pyrophosphate gel electrophoresis and gel electrophoresis-derived enzyme linked immunosorbent assay (GEDELISA) of myosin isoforms. The antibodies used were monoclonals against heavy chains of slow and fast myosins and a polyclonal against foetal/embryonic myosin. In both muscles in the newborn kitten, there was a population of more mature fibres which stained strongly for slow but weakly for foetal/embryonic myosin. These fibres were considered to be primary fibres. They formed 4.8% of EDL fibres and 26% of soleus fibres at birth, and continued to express slow myosin in adult muscles. The less mature secondary fibres stained strongly for foetal/embryonic myosin, and these could be divided into two subpopulations; fast secondaries in which foetal/embryonic myosin was replaced by fast myosin, and slow secondaries in which the myosin was replaced by slow myosin. At 50 days the EDL had a large population of fast secondaries (83% of total fibres) and a small population of slow secondaries which gradually transformed into fast fibres with maturity. The vast majority of secondary fibres in the soleus were slow secondaries, in which slow myosin synthesis persisted in adult life. There was a restricted zone of fast secondaries in the soleus, and these gradually transformed into slow fibres in adult life. It is proposed that the emergence of primary fibres and the two populations of secondary fibres is myogenically determined.

Riley DA, Bain JLW, Ellis S, and Haas AL. Quantitation and immunocytochemical localization of ubiquitin conjugates within rat red and white skeletal muscles. ***J Histochem Cytochem*** **1988;36(6):621-632.** We employed solid-phase immunochemical methods to probe the dynamics of ubiquitin pools within selected rat skeletal muscles. The total ubiquitin content of red muscles was greater than that of white muscles, even though the fractional conjugation was similar for both types of muscle. The specificity for conjugated ubiquitin in solid-phase applications, previously demonstrated for an affinity-purified antibody against SDS-denatured ubiquitin, was retained when used as a probe for ubiquitin-protein adducts in tissue sections. Immunohistochemical localization revealed that differences in ubiquitin pools derived from the relative content of red (oxidative) vs white (glycolytic) fibers, with the former exhibiting a higher content of ubiquitin conjugates. Subsequent immunogold labeling demonstrated statistically significant enhanced localization of ubiquitin conjugates to the Z-lines in both red and white muscle fiber types.

Riley DA, Ellis S, and Bain JLW. Catalase-positive microperoxisomes in rat soleus and extensor digitorum longus muscle fiber types. ***J Histochem Cytochem*** **1988;36(6):633-637.** The size, distribution, and content of catalase-reactive microperoxisomes were studied cytochemically in slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), and fast-twitch glycolytic (FG) fibers of soleus and extensor digitorum longus (EDL) rat muscles. Fiber types were classified on the basis of mitochondrial content and distribution, Z-band widths, and myofibril size and shape. Microperoxisomes were generally located between myofibrils at the I-bands. The absence of crystalloid inclusions prevented positive identification of microperoxisomes in nonreacted and aminotriazole-inhibited muscles. EDL and soleus SO fibers

possessed the largest microperoxisomes, whereas FOG and FG fibers of the EDL contained small- to medium-sized microperoxisomes. Comparing either microperoxisome number per muscle fiber area or microperoxisome area per fiber area revealed significant differences between fiber types with this ranking: soleus SO > EDL SO > EDL FOG > EDL FG. The present observations demonstrate that the content of catalase-positive microperoxisomes is greatest in the oxidative muscle fiber types. These cytochemical findings account for the higher catalase activity in homogenates of soleus muscles as compared to that of EDL muscles, because the soleus contains more oxidative fibers than EDL.

Riley DA, Ellis S, Slocum GR, Satyanarayana T, Bain JLW, and Sedlak FR. Hypogravity-induced atrophy of rat soleus and extensor digitorum longus muscles. ***Muscle Nerve*** **1987;10:560-568.** Prolonged exposure of humans to hypogravity causes weakening of their skeletal muscles. This problem was studied in rats exposed to hypogravity for 7 days aboard Spacelab 3. Hindlimb muscles were harvested 12-16 hours postflight for histochemical, biochemical, and ultrastructural analyses. The majority of the soleus and extensor digitorum longus fibers exhibited simple cell shrinkage. However, approximately 1% of the fibers in flight soleus muscles appeared necrotic. Flight muscle fibers showed increased glycogen, lower subsarcolemmal staining for mitochondrial enzymes, and fewer subsarcolemmal mitochondria. During atrophy, myofibrils were eroded by multiple focal losses of myofilaments; lysosomal autophagy was not evident. Tripeptidylaminopeptidase and calcium-activated protease activities of flight soleus fibers were significantly increased, implying a role in myofibril breakdown. Simple fiber atrophy appears to account for muscle weakening during spaceflight, but fiber necrosis is also a contributing factor.

Riley DA, Ellis S, Slocum GR, Satyanarayana T, Bain JLW, and Sedlak FR. Morphological and biochemical changes in soleus and extensor digitorum longus muscles of rats orbited in Spacelab 3. ***Physiologist*** **1985;28(6,Suppl):S207-S208.** Flight rats were exposed to 7 days of hypogravity, and control animals remained on earth in simulated flight cages. Hindlimb muscles were harvested 12-16 hours postflight. Mean soleus fiber area decreased 35.8%. Extensor digitorum longus (EDL) fibers atrophied 24.9%. Most atrophic fibers were small versions of control fibers, indicating simple cell shrinkage. However, up to 1% of the fibers in flight soleus muscles appeared undergoing cell death. Also present were large round fragmented fibers possibly broken during the postflight exposure to terrestrial gravity or during tissue processing. Both the EDL and soleus muscles acquired fast histochemical properties, i.e., increases in alkaline myofibrillar ATPase, alpha glycerophosphate dehydrogenase, glycogen and a decrease in NADH dehydrogenase staining. Myofibrils of flight solei were eroded by multiple focal losses of myofilaments. Following flight, tripeptidylaminopeptidase and total calcium activated protease activities were significantly increased by 60% and 26% respectively. These two proteases possibly function in myofibril breakdown. The muscle fiber changes described herein cannot be attributed solely to hypogravity because of the long postflight exposure of the rats to terrestrial gravity.

Riley DA, and Fahlman CS. Colchicine-induced differential sprouting of the endplates on fast and slow muscle fibers in rat extensor digitorum longus, soleus and tibialis anterior muscles. ***Brain Res*** **1985;329:83-95.** The patterns of sprouting of motor endplates were examined in fast extensor digitorum longus and slow soleus muscles and in tibialis anterior muscles containing fast and slow muscle fiber types. A histochemical technique combining nerve silver impregnation and endplate cholinesterase staining was developed for this task. Temporal examination of the innervation was conducted 3, 7, and 10 days after either a 45 or 90 min application of the ipsilateral sciatic nerve with 5 mM colchicine. This dosage of drug did not cause detectable axon or muscle fiber degeneration, unlike 60 mM which was highly neurotoxic. At 3 days following treatment with the lower concentration, there were no significant differences in the percentages

of intranodal, preterminal and ultraterminal sprouts between the normal (non-treated), sham-treated, contralateral systemic-control and drug-treated groups of muscles. By 7 and 10 days, the muscles on the drug-treated side exhibited significant increases in the 3 types of sprouts. Collateral sprouting was uncommon: most outgrowths remained on the muscle fibers innervated by the parent axons. Endplates in the tibialis anterior muscles of the control and drug-treated groups were classified Complex, Intermediate or Simple according to the relative degrees of branching of the terminal arbors. The occurrence of endplate classes and muscle fiber types was correlated in the superficial and deep regions of this muscle. Complex endplates innervated fast glycolytic fibers, Intermediate endplates supplied fast oxidative glycolytic fibers, and Simple endplates served slow oxidative fibers. In response to colchicine, the endplates of the slow muscles sprouted more than those of fast muscles while the innervation of slow fiber types sprouted less than that of fast fiber types. Furthermore, intranodal sprouts were more prevalent in slow muscles and ultraterminal sprouts more numerous in fast muscles whereas intranodal sprouts predominated on fast fiber types and ultraterminal sprouts were characteristic of slow fiber types. These apparently contradictory results were reconciled when it was noted that soleus endplates were mostly Complex and Intermediate, and the extensor digitorum longus contained more Simple endplates. Thus, consistency of sprouting patterns among endplate types of the 3 muscles was recognized when the pre-existing branching patterns were considered. This indicated that the patterns of sprouting were determined by the motor neurons rather than the muscle fibers. The observed sprouting responses supported the hypothesis that colchicine treatment of motor axons caused muscle fibers to elaborate a diffusible sprout-inducing factor.

Riley DA, Ilyina-Kakueva EI, Ellis S, Bain JLW, Slocum GR, and Sedlak FR. Skeletal muscle fiber, nerve, and blood vessel breakdown in space-flown rats. ***FASEB J*** **1990;4:84-91.** Histochemical and ultrastructural analyses were performed postflight on hind limb skeletal muscles of rats orbited for 12.5 days aboard the unmanned Cosmos 1887 biosatellite and returned to Earth 2 days before sacrifice. The antigravity adductor longus (AL), soleus, and plantaris muscles atrophied more than the non-weight bearing extensor digitorum longus, and slow muscle fibers were more atrophic than fast fibers. Muscle fiber segmental necrosis occurred selectively in the AL and soleus muscles; primarily, macrophages and neutrophils infiltrated and phagocytosed cellular debris. Granule-rich mast cells were diminished in flight AL muscles compared with controls, indicating that mast cell secretion contributed to interstitial tissue edema. Increased ubiquitination of disrupted myofibrils implicated ubiquitin in myofilament degradation. Mitochondrial content and succinic dehydrogenase activity were normal, except for subsarcolemmal decreases. Myofibrillar ATPase activity of flight AL muscle fibers shifted toward the fast type. Absence of capillaries and extravasation of red blood cells indicated failed microcirculation. Muscle fiber regeneration from activated satellite cells was detected. About 17% of the flight AL end plates exhibited total or partial denervation. Thus, skeletal muscle weakness associated with spaceflight can result from muscle fiber atrophy and segmental necrosis, partial motor denervation, and disruption of the microcirculation.

Riley DA, Sanger JR, Matloub HS, Yousif NJ, Bain JLW, and Moore GH. Identifying motor and sensory myelinated axons in rabbit peripheral nerves by histochemical staining for carbonic anhydrase and cholinesterase activities. ***Brain Res*** **1988;453:79-88.** Carbonic anhydrase (CA) and cholinesterase (CE) histochemical staining of rabbit spinal nerve roots and dorsal root ganglia demonstrated that among the reactive myelinated axons, with minor exceptions, sensory axons were CA positive and CE negative whereas motor axons were CA negative and CE positive. The high specificity was achieved by adjusting reaction conditions to stain subpopulations of myelinated axons selectively while leaving 50% or so unstained. Fixation with glutaraldehyde appeared necessary for achieving selectivity. Following sciatic nerve transection, the reciprocal staining pattern persisted in damaged axons and their regenerating processes which formed neuromas within the proximal nerve stump. Within the neuromas, CA-stained

sensory processes were elaborated earlier and in greater numbers than CE-stained regenerating motor processes. The present results indicate that histochemical axon typing can be exploited to reveal heterogeneous responses of motor and sensory axons to injury.

Riley DA, and Slocum GR. Contraction-free, fume-fixed longitudinal sections of fresh frozen muscle. *Stain Technol* 1988;63(2):93-96. Contraction damage occurring when longitudinal frozen sections of fresh unfixed muscles are thawed on microscope slides has limited histological examination of this tissue mainly to cross sections. Longitudinally oriented sections are advantageous for investigating properties that vary along the length of the muscle fibers. A fume fixation technique has been developed for preventing contraction of thick longitudinal frozen sections. The technique is compatible with histochemical staining of enzymes.

Yip RK, and Riley DA. Effects of methylmercury on the motor and sensory innervation of the rat extensor digitorum longus muscle. *Environ Res* 1987;43:85-96. This histochemical study examined the effects of chronic methylmercury (MeHg) intoxication on the motor and sensory innervation of extensor digitorum longus muscles. Light microscopic examination of silver-stained axons in the intramuscular nerve bundles of MeHg-treated rats showed Wallerian-like degeneration and a reduction in the number of nerve fibers. Disrupted axons were predominantly sensory because 22.2% of spindle afferents (I_a) and 90.0% of Golgi tendon organ (I_b) sensory fibers were completely degenerated whereas less than 1% of motor endings were totally destroyed. Partial disruption occurred in the cholinesterase and motor terminals of 13.7% of endplates. Our results demonstrated greater vulnerability of sensory nerves than of motor nerves to MeHg-induced degeneration. Thus, the abnormal reflexes, ataxia, and muscle weakness following MeHg poisoning appear related to reduction of proprioceptive feedback from muscles and tendons in addition to the documented lesions in the central nervous system.

HEMATOLOGY AND IMMUNOLOGY

Bechler B, Cogoli A, and Mesland D. Lymphozyten sind schwerkraftempfindlich (Are lymphocytes sensitive to gravitational forces?). *Naturwissenschaften* 1986;73:400-403. Two experiments dedicated to the study of human lymphocyte activation by the mitogen concanavalin A (Con A) in microgravity were performed. All cultures in micro-*g* had a corresponding inflight 1-*g* control in a reference centrifuge. In all cultures kept under micro-*g* conditions, activation by Con A is depressed by more than 90% as compared to the control. Activation of lymphocytes from the crew members is markedly depressed in the 1-*g* inflight as well as in the samples drawn 1 h after landing, as compared to the preflight values. Activation recovers to preflight level between 7 and 13 days after landing.

Cogoli A, Bechler B, Müller O, and Hunzinger E. Effect of microgravity on lymphocyte activation. In: BIORACK ON SPACELAB D1 (Eds.) Logdon N, and David V. Noordwijk: ESA Publications Division (ESA SP-1091), 1988;89-100.

Cogoli A, and Tschopp A. Effect of spaceflight on lymphocyte stimulation. *Physiologist* 1980;23(6,Suppl):S63-S66.

Cogoli A, and Tschopp A. Lymphocyte reactivity during spaceflight. *Immunol Today* 1985;6(1):1-4. Over twenty years of spaceflight have demonstrated that man can easily survive and work in weightless conditions. However, a number of physiological changes may affect crew performance in space. Besides the well known disturbances of the vestibular and cardiovascular systems, bone demineralization, and decrease of erythrocyte mass, certain immunological alterations have been observed in space crews after flight. One of them - the reduction of lymphocyte reactivity to mitogens - was the subject of our investigations during the flight of Spacelab 1 from 28 November - 8 December 1983.

Dunn CRD, Johnson PC, and Lange RD. Regulation of hematopoiesis in rats exposed to antiorthostatic hypokinetic/hypodynamia: II. mechanisms of the "anemia". *Aviat Space Environ Med* 1986;57(1):36-44. Results are presented which demonstrate a close similarity between the ability of hypokinetic/hypodynamia and orthostatic hypokinetic/hypodynamia to induce anemia in laboratory rats. The "restraint anemia" (whether mediated directly by reduced activity or indirectly by possible changes in blood circulation or in altered weight-bearing capacity of the skeleton) was largely due to reduced food and/or water consumption and displayed the classical symptons of inadequate nutrition, i.e. decreased serum erythropoietin (Ep) titers and reduced Ep sensitivity of hematopoietic tissue. Only changes in red blood cell (RBC) clearance were unique to the head-down (antiorthostatic) posture. During suspension, RBC clearance was reduced and then accelerated when suspension was terminated or the cells transfused into a normal environment. Changes in RBC clearance were due to both cell-associated and cell-independent factors and may be related to the alterations in RBC survival seen in rats during or immediately after space flight. In both suspension and weightlessness, these changes were limited to alterations in the force and/or direction of the gravity vector.

Dunn CDR, Johnson PC, Lange RD, Perez L, and Nessel R. Regulation of hematopoiesis in rats exposed to antiorthostatic, hypokinetic/hypodynamia: I. model descripiton. *Aviat Space Environ Med* 1985;56(5):419-426. This paper provides baseline information regarding the regulation of hematopoiesis in antiorthostatic, hypokinetic/hypodynamic ("suspended") laboratory rats. The object of the study was to compare the hematological effects of suspension with those seen following space flight in man and/or rats.

Observed in man after exposure to microgravity and in the suspended rats was a reduced red blood cell mass, suppressed erythropoiesis, a transient increase in hematocrit due to a reduction in plasma volume, a post-exposure hematocrit decrease, a weight loss (or failure to thrive) and a reduction in food and water consumption. A rightward shift in the oxyhemoglobin dissociation curve, observed in the rat "model", has been predicted to occur during manned space flight but has not yet been measured. Suppression of hematopoiesis is a common feature of rats during both space flight and suspension. Platelet counts showed no significant change in rats after suspension or in man during space flight. Unlike man in space but similar to space flight-exposed rats, no significant change in leukocyte number or reactivity to PHA *in vitro*, or in red blood cell shape distribution were observed in the suspended rats. At least in a gross sense, the rat "model" seems to reproduce many of the known hematological effects of space flight and offers promise as a l-g analog for understanding hematopoietic effects similar to those found in space flight.

Gmünder FK, Lorenzi G, Bechler B, Joller P, Müller J, Ziegler WH, and Cogoli A. Effect of long-term physical exercise on lymphocyte reactivity: similarity to spaceflight reactions. ***Aviat Space Environ Med*** **1988;59(2):146-151.** The response of critical immunological parameters in seven athletes to the sustained physical stress of marathon running was assessed. Variables analysed were the responsiveness of lymphocytes (measured as mitogenic response to concanavalin A), the numbers of lymphocytes, their subsets, and leukocyte numbers. In addition, blood levels of cortisol, epinephrine, and norepinephrine were determined. After the run, lymphocyte responsiveness was severely depressed to 1-70% of the resting values, even though the lymphocyte counts did not change. Leukocyte counts were elevated 2.8-fold. No dramatic changes were found within the lymphocyte subsets, although an increase in pan T-cells and the helper/inducer subset 2 d after the run was significant. In addition, the numbers of B-cells decreased significantly. No change was observed within the suppressor/cytotoxic subset. Cortisol increased 2.1-fold, epinephrine 3.2-fold, and norepinephrine 2.7-fold. All these parameters returned to baseline values within 2 d. These data were compared with data obtained during and after spaceflight. We conclude that prolonged physical stress of marathon running induces changes in immunological responsiveness that are strikingly similar to those arising from the stress of spaceflight.

Lange RD, Andrews RB, Gibson LA, Congdon CC, Wright P, Dunn CDR, and Jones JB. Hematological measurements in rats flown on Spacelab shuttle, SL-3. ***Am J Physiol*** **1987;252:R216-R221.** Previous studies have shown that a decrease in red cell mass occurs in astronauts, and some studies indicate a leukocytosis occurs. A life science module housing young and mature rats was flown on shuttle mission Spacelab 3 (SL-3), and the results of hematology studies of flight and control rats are presented. Statistically significant increases in the hematocrit, red blood cell counts, and hemoglobin determinations, together with a mild neutrophilia and lymphopenia, were found in flight animals. No significant changes were found in bone marrow and spleen cell differentials or erythropoietin determinations. Clonal assays demonstrated an increased erythroid colony formation of flight animal bone marrow cells at erythropoietin doses of 0.02 and 1.0 U/ml but not 0.20 U/ml. These results agree with some but vary from other previously published studies. Erythropoietin assays and clonal studies were performed for the first time.

Lange RD, Andrews RB, Gibson LA, Wright P, Dunn CDR, and Jones JB. Hematological studies on rats flown on shuttle flight SL-3. In: REGULATION OF ERYTHROPOIESIS (Eds.) Lange ED, Tavassoli M, and Ascensao JL. New York: PMA Publishing Co., 1988;455-466. Astronauts who have flown in microgravity have experienced a loss of red blood cell mass. The pathogenesis of this anemia of space flight has not been ascertained, although it is probably multifactorial. A few experiments have been conducted on laboratory animals which demonstrate some of the same changes found in human astronauts.

From 04/29/85 to 05/06/85, 24 white rats were flown on the SL-3 mission of the shuttle Challenger. Although this was primarily an engineering flight, these animals were studied upon return. The results of hematologic studies are presented in this chapter.

Lange RD, Andrews RB, Gibson LA, Wright P, Dunn CDR, and Jones JB. Hematologic parameters of astrorats flown on SL-3. *Physiologist* 1985;28(6,Suppl):S195-S196. Hematologic studies were performed on a group of large and small rats which were sacrificed after flying in life sciences shuttle engineering flight SL-3. The results are presented on flight (F) and control (C) 200 gm rats.

The small flight animals demonstrated a significant increase in hematocrits, red blood cell counts, hemoglobins and peripheral blood percentages of neutrophils as well as a decrease in percentage of lymphocytes. Erythropoietin (Ep) determinations were similar for the two groups as were the bone marrow and spleen differential counts. In vitro cultures for erythroid colonies of bone marrow showed that in response to different doses of Ep, in all cases where differences were statistically significant, the F rats had increased colony counts.

The changes in red cell parameters could be caused by a decrease in plasma volume. However, no isotopic studies were possible on this flight and this lack points up the need for such studies to determine the red cell mass and plasma volume.

Leach CS, Chen JP, Crosby W, Johnson PC, Lange RD, Larkin E, and Tavassoli M. Hematology and biochemical findings of Spacelab 1 flight. In: REGULATION OF ERYTHROPOIESIS (Eds.) Zanjani ED, Tavassoli M, and Ascensao JL. New York: PMA Publishing Corp., 1988;415-453. The most consistent finding in studies of the influence of space flight on the hematologic system of man has been a significant reduction in the circulating red cell mass (RCM). This phenomenon has been observed in the American Gemini, Apollo, Skylab, and Apollo-Soyuz Test Project missions, and Soviet Soyuz-Salyut missions. Data from the Skylab flights suggest that suppression of normal erythropoiesis may be a cause of red cell mass reduction found after space flight.

An experiment conducted on the 10-day Spacelab 1 mission aboard the ninth Space Shuttle flight in November to December 1983 was designed to measure factors involved in the control of erythrocyte turnover - particularly erythropoiesis - in man which might be altered soon after the beginning of exposure to weightlessness. Many of these hematological and biochemical parameters have not previously been measured in blood specimens collected during space flight.

Lorenzi G, Fuchs-Bislin P, and Cogoli A. Effects of hypergravity on "whole-blood" cultures of human lymphocytes. *Aviat Space Environ Med* 1986;57(12):1131-1135. The purpose of this paper is to present a detailed description of the effects of hypergravity on the mitogenic response of human lymphocytes to concanavalin A. The effect on cultures of lymphocytes isolated from peripheral blood are compared with those on whole-blood cultures obtained by diluting fresh blood with culture medium 1:10. Whole-blood cultures of lymphocytes from crew members will be investigated inflight on the Spacelab mission D-1 in 1985 and SLS-1 in 1987. In hypergravity there is an increase in lymphocyte activation of up to 500%. A similar increase can be induced by pre-incubating the cultures in hypergravity prior to exposure to concanavalin A at 1 G. The effect is less evident in cultures of isolated lymphocytes. The influence of autologous plasma and erythrocytes has also been investigated. Plasma and hypergravity have a synergistic and positive effect on lymphocyte activation, i.e. cultures of separated lymphocytes show the highest activation when incubated at 10 G and supplemented with autologous plasma. Conversely, erythrocytes depress lymphocyte activation.

Nachtman RG, Driscoll TB, Gibson LA, and Johnson PC Jr. Commercial over-the-needle catheters for intravenous injections and blood sampling in rats. ***Lab Anim Sci*** **1988;38(5):629-630.**

Nachtman RG, Dunn CDR, Driscoll TB, and Leach CS. Methods for repetitive measurements of multiple hematological parameters in individual rats. ***Lab Anim Sci*** **1985;505-508.** Methods have been developed which permit frequent repetitive blood sampling of rats without perturbing physiological parameters of interest. These techniques allow a comprehensive hematological study over several weeks, in individual rats, thus permitting full documentation of selected parameters during growth and development.

GRAVITATIONAL PHYSIOLOGY

Leonard JI, Leach CS, and Rambaut PC. Quantitation of tissue loss during prolonged space flight. ***Am J Clin Nutr*** **1983;38:667-679.** An analysis of data from the three Skylab missions was performed to assess the lean body mass (LBM) and fat components of inflight body weight loss. Six methods for determining LBM were employed based on changes in total body water, total body potassium, nitrogen balance, potassium balance, and stereophotometric-body density. Those based solely on body potassium, and potassium and nitrogen balances (when expressed as shifts from preflight control), consistently overestimated LBM loss unless appropriate corrections were made. The average results from the various methods indicated that of a mean inflight total body weight loss of 2.7 ± 0.3 kg (SD) for all nine crewmembers, more than half (1.5 ± 0.3 kg) can be attributed to loss of LBM (including 1.1 kg body water), the remainder (1.2 ± 0.3 kg) being derived from fat stores. The reduction of LBM appeared to be complete after the first month of flight and thereafter was largely independent of mission duration, diet, and exercise.

Ross MD. The influence of gravity on structure and function of animals. ***Adv Space Res*** **1984;4(12):305-314.** Gravity is the only environmental parameter that has remained constant during the period of evolution of living matter on Earth. Thus, it must have been a major force in shaping living things. The influence of gravitational loading on evolution of the vertebrate skeleton is well recognized, and scale effects have been studied. This paper, however, considers in addition four pivotal events in early evolution that would seem to have been significant for the later success and diversification of animal life. These are evolution of the cytoskeleton, cell motility (flagellae and cilia), gravity detecting devices (accelerometers), and biomineralization. All are functionally calcium dependent in eukaryotes and all occurred or were foreshadowed in prokaryotes. A major question is why calcium was selected as an ion of great importance to the structure and function of living matter; another is whether gravity played a role in its selection.

Spangenberg DB. Statolith formation in Cnidaria: effects of cadmium on Aurelia statoliths. ***Scan Electron Microsc*** **1986;4:1609-1618.** Statolith formation in Cnidaria was reviewed with an emphasis on Aurelia statoliths. The review provides information on the chemical composition, mechanisms of initiation of mineralization, and effects of environmental factors on Cnidarian statolith formation. Environmental factors discussed include modified sea water ingredients, X-irradiation, clinostat rotation, and petroleum oil ingredients. A detailed account of the effects of cadmium on mineralization and demineralization of Aurelia statoliths is given. Cadmium at dosages of 2 to 4 μM significantly reduces statolith numbers in developing ephyrae. At a dosage of 3 μM, cadmium accelerates statolith loss in unfed ephyrae studied at 4 and 8 days following ephyra release from strobilae. Cadmium, therefore, is shown to reduce statolith numbers in developing ephyrae and to cause greater reduction of statolith numbers in unfed ephyrae after 4 and 8 days than occurred in controls. Supplementation of Cd^{2+}-containing artificial sea water (ASW) with calcium (3X and 5X ASW calcium content) results in higher numbers of statoliths at day 4 as compared with cadmium-treated ephyrae. At 8 days only the 5X calcium supplemented ASW is effective in enhancing statolith numbers in Cd^{2+}-treated ephyrae. These results suggest that cadmium competes in some manner with calcium at the mineralizing sites of Aurelia.

Spangenberg DB. Rhopalium development in *Aurelia aurita* ephyrae. ***Hydrobiologia*** **1991;IN PRINT.** Rhopalia of developing ephyrae were examined using the SEM and TEM at 24 h intervals following strobilation induction. Kinocilia are shorter in the ephyrae stage than in polyps. A few ephyrae-type kinocilia are found in rhopalia as early as 24 h after induction, before a distinct rhopalium is seen. By 72 h, the shorter kinocilia predominate and are almost as numerous as in ephyrae (120 h). Many of the kinocilia are associated with mechanoreceptor cells (MR) found in the rhopalia. These MR cells are compared to those reported for medusae. Although newly-released ephyrae lack a touch plate, the MR cells in their rhopalia along with the statocyst and neuromuscular system apparently enable these organisms to detect and respond to gravity.